University of Alberta

Vanadium Speciation Using HPLC-ICPMS and Its Environmental Applications

by

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Whatever is true, whatever is noble, whatever is right, whatever is pure,

whatever is lovely, whatever is admirable –

if anything is excellent or praiseworthy, think about such things.

Philipians 4:8 (NIV)

To my parents and Dabo –

For your amazing love, endless patience and your faith in me

have made me better than I thought possible

Abstract

Vanadium is an environmental contaminant that exists in the (III), (IV) and (V) oxidation states in nature. The toxicity of vanadium depends on its concentration and oxidation state, with the V(V) species being the most toxic.

In this research, a high performance liquid chromatography inductively coupled mass spectrometry method was developed for the speciation of V using a strong anion exchange column. The buffer was optimized to contain 3% acetonitrile, 2 mM EDTA and 80 mM NH_4HCO_3. The complexes $[VY{\cdot}H_2O]^-$, $[VOY]^{2-}$ and $[VO_2Y]^{3-}$ had detection limits of 0.6, 0.7 and 1.0 µg/L, respectively. They were separated in less than 6 min with resolution of at least 3.8.

To test the robustness of the method, it was applied to coke water samples and to *Shewanella putrefaciens* CN32 samples incubated with V(V). In the coke water samples, V(IV) and V(V) were found, with V(V) being the more common species. For the first time, the bacterium was found to be able to reduce V to V(IV) and even to V(III).

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LIST OF ABBREVIATIONS

ACN	acetonitrile
BDL	below detection limit
CUR	curtain gas
DIW	deionized water
DP	declustering potential
EC	electrical conductivity
ESI-MS	electrospray ionization mass spectrometry
FP	focusing potential
GS1	sheath gas
HPLC	high performance liquid chromatography
ICPMS	inductively coupled mass spectrometry
IS	ionspray voltage
SAX	strong anion exchange
SMV	sodium metavanadate
TOF	time-of-flight
V	Vanadium
V(III)	inorganic Vanadium (III)
V(IV)	inorganic Vanadium (IV)
V(V)	inorganic Vanadium (V)
$[VY{\cdot}H_2O]^-$	V(III)-EDTA
$[VOY]^{2-}$	V(IV)-EDTA
$[VO_2Y]^{3-}$	V(V)-EDTA

Chapter 1. Introduction

1.1 Background of Vanadium

The name "vanadium" has a romantic history. Due to its beautiful multicolored compounds, the Swedish scientist Nils Sefstrom named the element he discovered in 1831 after the Scandinavian goddess of love, youth and beauty, Vanadis (1). As a polyvalent transition metal, vanadium displays a broad range of beautiful hues, from yellow to green and from red to black.

Vanadium (V) in its pure state is a soft and ductile metal that can be added in small amounts (up to 5%) to steel. This results in a metal that is more durable, lasts longer and has improved mechanical properties without heat treatment (2). Besides being used in processing steel, vanadium is also used in the production of dyes, chemicals, polymers, ceramics, catalysts and electronics.

In the natural environment, vanadium constitutes about 0.01% of the crust of the earth. It is also found in small amounts in air, water and food. In food, it is present in vegetable oil, grains, skim milk and lobster at concentrations around 1 μg/kg. Normally, the amount of V ingested daily is 10 – 60 μg. Once it is ingested, absorbed V mainly accumulates in the bone, liver, kidney, spleen and testes, with the highest concentrations in the liver and bone (3). Around 90% of V is excreted in urine whereas only a small amount (10%) of ingested V is absorbed. Although urinary vanadium concentrations are generally higher for those who are most heavily exposed, urine is not a good biomarker for V exposure (4), as found by recent studies on cattle farmed near a vanadium mine (5).

1.2 Relevance to Human Health

In biological systems, vanadium is found to play a role in cellular metabolism, formation of bones and teeth, reproduction and growth when it is present at μg/kg concentrations. It is also believed to be involved in the metabolism of glucose and lipids, but its biochemical function in the human body is poorly understood. At these low concentrations, V is inductive to normal cell growth. In fact, studies in goats revealed that vanadium deficiency causes irreversible bone deformity, depressed milk production, increased abortion rate and increased infant mortality rate (6). However, no related studies were done in humans.

1.2.1 Medicinal properties

Vanadium is not only important for proper biological function, but it also has medicinal uses. In the 1920s, vanadium compounds were used to supply the body with oxygen in anemic patients. It was also used as an antiseptic in acute infections such as influenza and pneumonia. In pulmonary tuberculosis, V compounds are said to increase appetite, improve nutrition and enhance the immune system. In chronic rheumatism and rheumatoid arthritis, V compounds lead to symptomatic relief. In addition, they act as oxidizers and stimulate nutritive processes (7) and thus prevent cancer.

But perhaps what vanadium is most renowned for in the medical community is its effectiveness against diabetes in vitro and in vivo (8). It has been found that several bisperoxovanadium compounds (9), especially bis(maltolato)oxovanadium (IV), are effective insulin mimics (10). These compounds enhance insulin sensitivity by inhibiting the protein tyrosine phosphate and activating the insulin receptor kinase. The results are

improved glucose tolerance, lowered blood glucose levels and corrected metabolic deficiencies in several species of diabetic animals and in human patients.

In addition to possessing anti-diabetic properties, the recently discovered vanadium drug Metvan™ is effective in inducing apoptosis in primary leukemia cells from patients with this disease (11). The pentavalent diammonium oxytartarovanadate has been studied for its cholesterol-reducing effects (12) and two pentavalent vanadium/oxovanadium complexes possess both anti-HIV and spermicidal activities at low μM concentrations (13). These properties make vanadium a very interesting element to study.

Despite their many advantages, V-containing compounds are not marketed as commercial drugs due to the concern of long-term tissue accumulation in the body that might lead to adverse health effects. The controversial debates over their toxicity have hindered research in the area of drug development.

1.3 Toxicity of Vanadium

Despite its many potential benefits as a multi-functional drug, there is growing evidence for mutagenic, genotoxic and carcinogenic potential for V salts in cultured mammalian cells (14). It is truly a case of dose determining toxicity. When V is incorporated into cells at μg/L concentrations, it is beneficial for cell growth. However, when it is present in cells at mg/L levels, it is a toxicant.

1.3.1 Human toxicity

A number of studies have been done with animals and humans exposed to V via inhalation and oral ingestion at mg/kg levels. Signs of toxicity included irritated mucous

and lung, lethargy and diarrhea. In addition, studies performed on pregnant diabetic rats showed that V(V) had a significant negative impact on the rate of conception and fetal development when administered at 250 mg/L (15).

Humans can be exposed to high concentrations of vanadium by cleaning oil-fired boilers and furnaces, by handling catalysts in chemical manufacturing plants and during the production of vanadium-containing pigments for the ceramics industry (16).

In a study done in 1967, nine healthy volunteers were divided in groups of three and were exposed to 0.1, 0.25 and 1 mg/m^3 of V_2O_5 for 8-h. An increasing concentration of V exposure correlated to coughs of increasing severity and duration with excessive production of mucus (17). In another study, diammonium vanadotartrate was administered orally at a larger dosage for a longer period of time, resulting in persistent upper abdominal pain, anorexia, nausea and weight loss in some patients. Some patients developed "green tongue" and pharyngitis with marginal ulceration of the tongue. The observation of the green tongue could be due to the formation of V(III) and V(IV) complexes in the mouth by bacteria and ptyalin (18).

1.3.2 Environmental toxicity

Even though the toxicity of V to humans has vital importance, the research carried out in this thesis focuses on V in the environment. Only a few studies have been reported regarding the hazardous effects of V to the environment, one of which was associated with a recent die-off of Canada geese. V(V) was found to be responsible for this incident that occurred at a petroleum refinery fly ash pond in Delaware (19). In studies following this event, V_2O_5 and Na_3VO_4 were tested to be moderately toxic to mallard drakes, a model species used extensively in wildlife toxicology. The LD_{50} for a 7-d oral dose trial

with was estimated to be 113 mg/kg of body weight for V_2O_5 and 75.5 mg/kg for Na_3VO_4, respectively. Canada geese were more susceptible to Na_3VO_4, with a LD_{50} of 37.2 mg/kg.

In aquatic organisms such as the adult American flagfish, the 96-h LC_{50} value was concluded to be 11.2 mg/L whereas the chronic toxicity was judged to be about 0.08 mg/L (20) when vanadium pentoxide was administered.

The concentration of V in plants is only one tenth of its concentration in soil. The metal is most concentrated in the roots and its concentration decreases towards the aerial part of the plant. It was also found that ten to 20 mg/L of aqueous vanadium stunts the growth of most plants (21). However, traces of it (1–2 mg of VO^{2+} or VO_3^-) were shown to promote chloroplast development and oxygen production (22) even though V has not been demonstrated to be an essential element for higher land plants.

1.4 Speciation of Vanadium

Vanadium has one of the most complex aqueous chemistries of any metal. Theoretically, it can exist in six oxidation states from -1 to +5. In the natural environment, it is usually found in the oxidation states (IV) and (V), and sometimes in (III). In most cases, V(V) is the most common species found in the environment because it is stable under oxic conditions. Dissolution of V(III) and V(IV) from minerals into water will quickly oxidize V to V(V) (16).

1.4.1 Vanadium (V)

The solution chemistry of V(V) is very complicated. At low pH, V(V) exist as VO_2^+. At low concentrations and neutral pH, V(V) exists mainly as the monomer $H_2VO_4^-$. This monomer is in a number of equilibria with the vanadate dimer ($H_3V_2O_7^-$),

Page 6 has been removed due to copyright restrictions.

The information removed was Fig 1-1. Predominate species of V(V) across different pH

Source: Michibata, H. and Kanamori, K. Selective Accumulation of Vanadium by Ascidians from Sea Water In *Vanadium in the environment, Part I. Chemistry and Biochemistry,* Nriagu, J.O., Ed.; John Wiley & Sons, Inc.: New York, **1998**; pp.217-249.

1.4.2 Vanadium (IV)

The V(IV) species predominates in reducing environments such as tissues in organisms and when pH is low. At pH < 2, V(IV) exist as VO^{2+}, or the hydrated form $VO(H_2O)_5^{2+}$. As pH increases above 4, this species is protonated to form $VOOH^+$. If the concentration is higher than 10^{-7} M, the dimer $(VOOH)_2^{2+}$ forms. At pH 7, V(IV) precipitates to give $VO(OH)_2$. Above pH 11, the precipitate dissolves to form $VO(OH)_3^-$. As pH becomes even higher, vanadate (VO_4^{3-}) is formed (26).

As early as 1911, sea squirts known as ascidians have been known to accumulate large residues of V(IV) in its blood cells from ocean waters (25). They accumulate V as V(IV) and these blood cells are known as vanadocytes. Amavadine is a natural product found in mushrooms containing V(IV) as an eight-coordinate complex. This complex is the most stable V(IV) complex known (4). In petroleum, V is present almost exclusively as V(IV) in very stable porphyrin and non-porphyrin complexes (27). In fact, about 50% of vanadium in oil is in the form of the vanadyl porphyrin complex (28).

1.4.3 Vanadium (III)

In reducing conditions, V(III) is also present in addition to V(IV) and V(V). In aqueous solution, V(III) exists as $[V(H_2O)_6]^{3+}$, $[V(OH)(H_2O)_5]^{2+}$, $[V(OH)_2(H_2O)_4]^+$ and $[V_2O(H_2O)_{10}]^{4+}$. When concentrations are high, trimers and tetramers of V(III) are formed (29). In solution, V(III) is a strong reducing agent and slowly attacks water, resulting in the production of V(IV) and hydrogen gas (30):

$$2V^{3+} + 2H_2O \rightarrow 2V^{4+} + 2OH^- + H_2\,(g)$$

V(II) and lower oxidation states of vanadium are too unstable in solution and are almost never observed.

1.5 Motivations of Current Research

Besides having different chemical properties, different species of vanadium have different toxicities. V(V), or vanadate, has a greater effect on phosphorylation reactions compared to the other oxidation states of V. Phosphorylation reactions are ubiquitous in biological systems and vanadate could interfere with them in a significant way. This is at least due in part to vanadate's structural similarity to phosphate (VO_4^{3-} vs PO_4^{3-}) under various conditions. The two ions have similar pKa values, electronic and structural properties (24), allowing vanadate to bind to many active sites of proteins to which phosphate would otherwise bind. It is believed that the more toxic properties of V(V), compared to V(IV) and V(III), are due to its similarity to phosphate.

An example that demonstrates the toxicity of V(V) is in its effect on Na/K-ATPase. The Na/K-ATPase is an enzyme that is essential for biological systems to function normally. Tests performed on yeast cells have proved that the V(V) ion is a strong inhibitor of the enzyme, whereas V(IV) appears to be a weaker inhibitor (31). In fact, 40 mM of Na_3VO_4 inhibited the enzyme's activity by 50% (32). This indicates that V(V) is more toxic than V(IV) to biological systems.

Besides inhibiting Na/K-ATPase, vanadate is also found to inhibit red cell Ca^{2+}-ATPase. This inhibition process is enhanced by a number of the enzyme's natural activators: Mg^{2+}, K^+, Na^+ and calmodulin, with K^+ and Na^+ exerting the greatest effects (33). Vanadate also inhibits Ca-dependent dynein and myosin ATPases, as well as phosphatases, kinases and ribonucleases (34). In addition, V(V) inhibits the processes of DNA synthesis and DNA repair by affecting some mammalian DNA-dependent polymerase and terminal deoxynucleotidyl transferase. Interestingly, in the same study,

oxy-vanadium (IV) was found to be less effective in inhibiting the nucleotidyl transferase enzyme (34).

Studies have corroborated the carcinogenic potential of V(V), but not of V(IV) in BALB/3T3 mouse embryo fibroblast cultures. Among these cultures, ammonium metavanadate tested positive for transforming the morphology of the cell, whereas vanadyl sulfate did not test significantly positive at doses from 3×10^{-7} M to 1×10^{-5} M. In the same study, colony-forming efficiency data revealed that V(V) is more cytotoxic to fibroblast cells than V(IV) at doses around 10^{-5} M (35).

From Table 1-1, V(V) in the form of V_2O_5 has a lower LD_{50} value than $VOSO_4 \cdot 5H_2O$ (36). In a separate study, the results again showed that V(V) is more toxic than V(IV) (37).

Table 1-1. LD_{50} values of different oxidation states of V in animal oral studies

Chemical	Species	LD_{50} (mg/kg)
V_2O_5	V(V)	64–117
$VOSO_4 \cdot 5H_2O$	V(IV)	467
Na_3VO_4	V(V)	32.5–40.5
$VOSO_4 \cdot 2H_2O$	V(IV)	87.8–103.7

The data in Table 1-1 demonstrate that in general, as the oxidation state of V increases, toxicity also increases. The dependence of the toxicity of vanadium on its oxidation state and concentration makes it important to develop a method that is efficient in separating and quantifying the different forms of vanadium in samples.

1.6 Review of Related Work

There is not a plethora of work being done in the area of vanadium speciation, but researchers in the past have taken a number of creative approaches to determine the two most prominent species of vanadium, V(IV) and V(V).

1.6.1 Earlier techniques

One of the earlier studies was performed using energy-dispersive X-ray fluorescence spectrometry (EDXRF). V(V) and V(IV) were precipitated with diethyldithiocarbamate (DDTC) at pH 1.8 and 4, respectively. The precipitates were each quantified with EDXRF (38).

Another technique that enabled the determination of V(IV) in the presence of excess V(V) was decribed by Itabashi et al (39). This is an indirect photometric method with a continuous-flow injection analysis. This technique involved the reduction of copper (II) by vanadium (IV) in the presence of neocuproine. By determining the copper(I)-neocuproine complex, V(IV) could be indirectly measured (39).

With heightened awareness of vanadium in the natural environment, more practical techniques were needed to separate both V species simultaneously. Because their concentrations in natural waters are usually very low, several techniques were developed to preconcentrate the V ions using chelating resins. In the work of Hirayama et al. (40), a two-column system was used to preconcentrate and separate V(IV) and V(V). The first column consisted of ethylenediamine bonded to chelating functional group immobilized silica gels (ED-CIS) and the second consisted of silica gel bonded with ethylenediaminetriacetate (ED3A-CIS). In the pH range 2.5–3, the former retained the

V(V) ion and the latter retained both the V(IV) and V(V) ions. The two species were completely separated and determined, even when V(V) was present at ten times the concentration of V(IV). The advantage of this technique is that it allows analysis in the field with a portable unit, so that a large volume of water can be sampled.

The disadvantage of this technique is that the V(IV) and V(V) ions, VO^{2+} and $H_2VO_4^-$, are highly unstable and they can interconvert between each other depending on slight pH or oxygen content changes. Even though an in situ analysis might be possible, it still cannot provide a totally accurate determination of the species.

In a more recent work done by Pyrzyńska and Wierzbicki (41), the cationic cellulose sorbent CellexP was used to preconcentrate and absorb both V(IV) and V(V) on a column. V(IV) was selectively eluted with cyclohexanediamine tetraacetic acid (CDTA) whereas V(V) was later eluted with EDTA. The species were determined by atomic absorption spectroscopy (AAS). However, this method does not separate V(IV) from the matrix interferences, thus making quantification impossible (41).

1.6.2 Reversed phase chromatography

A more popular technique for the speciation of vanadium has been developed using reversed phase chromatography with tetrabutyl ammonium (TBA) as the ion pairing agent.

In one of the earlier studies (42), V(V) was chelated with the azo dye 2-(8-quinolylazo)-5-(dimethylamino)phenol (DMQAP) in a pre-column to form the neutral complex [VO_2(DMQAP)]. This derivatized vanadium interacts with the ion pair agent TBA and is separated from other metals present in coal fly ash on a C18 column. The eluent consisted of acetonitrile (ACN), TBA and water (42).

A very nice method was developed by Jen and Yang (43). A C8 column was used for reverse-phase ion-pair separation of V(IV) and V(V) EDTA complexes, which carry -2 and -3 charges, respectively. TBA ion pair with the EDTA complexes, forming neutral entities that interact with the nonpolar stationary phase to different extents. The V complexes were eluted with 12% ACN, 50 mM TBA and 2 mM EDTA at pH 6. The separation was done in 20 min with a resolution of about 4.7. The resolution was calculated based on the retention times and peak widths as in Equation 1-1,

$$Rs = 2\,(t_2 - t_1) / (t_{w1} + t_{w2}) \qquad \text{Eqn 1-1}$$

where t_2 and t_1 are the retention times of the second eluting peak and the first peak, respectively. The peak widths of the first eluting peak and the second are represented by t_{w1} and t_{w2}, respetively.

The detection limit was 50 μg/L for each species. When this method was applied to leachate samples from an oil refining waste site, serious peak tailing occurred. To resolve this, ACN was removed from the eluent and sodium acetate added. As a result of these changes, the analysis time increased to 35 min (43).

A few years after Jen and Yang (43) published their results, Wann and Jiang (44) improved the limits of detecting V to 0.025 μg/L for V(IV) and 0.041 μg/L for V(V) using an inductively coupled plasma mass spectrometer (ICPMS) as the detector instead of UV. Their efforts also reduced the analysis time to only 4 min and separated the two V species from the ClO^+ interference. However, resolution between the two species was only about 1.3. Repeatability of the peak heights was 2.3% and 5.6% for V(IV) and V(V), respectively, which is slightly high, especially for V(V) (44).

The most recent reversed phase chromatography technique was developed by Colina et al (45). The mobile phase they employed consisted of 60 mM ammonium acetate, 10 mM TBA, 10 mM ammonium diphosphate and 2.5 mM EDTA at pH 6. The use of an organic modifier was avoided since it could reduce the sensitivity of detection. ICPMS was utilized as the detector for its high sensitivity. Even with these efforts to maximize sensitivity, the detection limits were still high, with V(IV) at 59.1 μg/L and V(V) at 113.1 μg/L. Under this system, the EDTA-complexed V peaks were broad and asymmetrical with a resolution of about 1.5 (45).

1.6.3 Ion chromatography

Besides reversed phase ion chromatography, another reported technique for V speciation was ion chromatography. Tomlinson et al. (46) used a strong cation exchange column to speciate V(IV) and V(V) using 2,6-pyridinedicarboxylic acid as the chelating agent at pH 4. Separation was made possible by the different degrees of association between the two species and the acid. The problem with this method is that there was on-column interconversion between the two species, causing them to be baseline unresolved. In addition, V(IV) appeared unstable in this elution system; it appeared as a very broad peak and its detection limit was high. As well, the V(IV) peak was asymmetrical and is unsuitable for quantification purposes (46).

The above example demonstrates that a strong cation exchange column can be used to elute cationic species. By the same principle, a strong anion exchange column can also be used to elute anionic complexes of V. The first column Hirayama et al. (40) used retained V(V) as $H_2VO_4^-$ between pH 2.5–3. Using a similar idea, Patel et al. (47) used a strong anion exchange column to selectively elute VO^{2+} first and preconcentrate

V(V) on column. V(V) was then eluted with NaOH and detected by flame atomic absorption spectrometry. Using this method, V(V) could be determined at the 50 μg/L level with a RSD of 3%.

Komarova et al. (48) separated V(IV)-EDTA and V(V)-EDTA monoperoxo complexes using anion exchange separation. V(IV)-EDTA, or $[VOY]^{2-}$ and the EDTA monoperoxo complex of V(V), or $[VO(O_2)Y]^{3-}$, were separated using Na_2CO_3 as the eluent. However, the two species had a resolution of only 0.8 and were not baseline resolved (48).

In the work done by Minelli et al. (49), a strong anion exchange column was used to trap both V species using Na_2EDTA. V(IV) was selectively eluted with an eluent containing Na_2EDTA, TBA and isopropanol. Detection was made using AAS with electrothermal atomization. The V(V) concentration was found by difference from the total [V]. The weakness of this procedure is that it does not allow a simultaneous determination of the two V species and is too lengthy for routine analysis (49).

A more efficient method was developed by Coetzee et al. (50). In this work, a short strong anion exchange guard column (AG5) was used to separate EDTA complexes of V in just 0.8 min with a resolution of about 2.5. However, the detection limits were 20 μg/L and 50 μg/L for V(IV) and V(V), respectively. The Dionex AG5 column requires a metal-free HPLC system, which could be expensive. Also, at high concentrations, the V(V) peak is broadened and cannot be baseline resolved from V(IV) (50).

Many improvements have been made in the area of vanadium speciation since the work was started a few decades ago. However, no method is perfect for routine and accurate analysis of V. With the discovery of vanadium as an emerging contaminant in

the environment, advances still need to be made in developing a simple method that is reliable for accurate and fast determination of V. To summarize, some of the disadvantages of the methods discussed above include lengthy procedure, high detection limit, high standard error, low resolution, long analysis time and instability of the V species on column. The work described in this thesis builds on what has been done before and aims to address these issues in developing an improved method that is applicable to some novel matrices.

1.7 Background on Samples Matrices

Once the HPLC-ICPMS method was developed, two different sample matrices were analyzed to test the applicability of the new method. The first type is coke pore water samples. These are water samples that have come in contact with coke, a coal-like byproduct of the oil refining process. The second type are *Shewanella putrefaciens* CN32 bacteria samples incubated with vanadium in the form of V(V).

1.7.1 Coke

The primary source of environmental contamination with vanadium is from the combustion of oil (16). Vanadium is present as a major component in crude oil where it is mostly present as an organometallic complex. When crude oil is upgraded, bitumen is extracted from it and some of the V is decomposed to its inorganic form which is soluble in water. Currently, two of the major commercial surface oil sands operations in the Fort McMurray region of Alberta are Syncrude Canada Ltd. and Suncor Energy Inc. At present, more than 6.8×10^4 tons of bitumen is upgraded daily, and this rate is expected

to grow dramatically over the next decade. Issues regarding the fate of V in the oil sands bitumen during the upgrading processes have been raised.

Generally, to obtain the usable form of oil, the highly viscous bitumen is converted to a less viscous sweet synthetic product that can then be further refined into petroleum products in an upgrading process. In commercial oil sands operations in Athabasca deposit in Alberta, the upgrader units employ high temperature cracking in coking and hydrocracker units. In this process, 10–12% of the feed bitumen is converted to coke, a coal-like byproduct which is not burned during processing. Coke is produced by spraying hot bitumen into a chamber of the coker filled with hot coke particles. When bitumen strikes a coke particle it is broken down into smaller molecules (51). The volatile lighter components vaporize and are recovered as petroleum products. Heavier residues that are not volatilized remain as mostly carbon with nitrogen, sulfur, trace metals and ash (51). At Syncrude alone, coke is produced at the rate of more than 4000 tons per day.

Even though they are both byproducts from upgrading bitumen, cokes from Suncor Energy Inc. and Syncrude Canada Ltd. are formed by two different processes. Suncor coke is produced by a delayed coking process where bitumen feed is pumped through the coking heater at 500°C (Figure 1-2). In this process, coke does not form until bitumen reaches the coke drum at a very high velocity. The heat of the coking heater is then turned off and the coke removed from the walls of the coke drum. This process is repeated in a cyclic operation and the coke produced is trucked to a stockpile whereas it is still hot (52). Suncor coke is stored in a dry form above ground and spontaneous fires have been observed to start from these cokes.

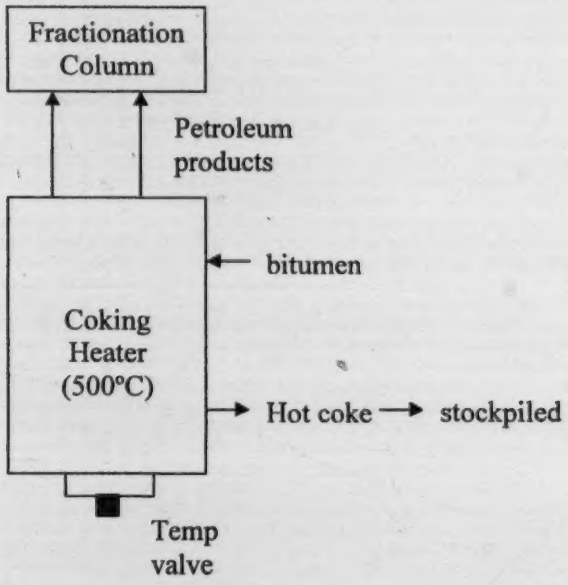

Figure 1-2. Simple schematic of Suncor delayed coking process

Syncrude coke is produced from a fluid coking process (Figure 1-3). Here bitumen is continuously sprayed into a bed of fluid coke maintained at 500°C in the coker where the coke formed is deposited on the surface of a seed particle (53). The heavier components that did not vaporize coat the coke particles and grow by accretion (54). The coke is then transferred to a reactor, where the coke particles are heated prior to being recycled back to the cokers. During this process, a fraction of the excess coke is withdrawn as waste, quenched with water and hydraulically transported to Mildred Lake Settling Basin (MLSB) beaches. In this thesis, two types of Syncrude coke were studied. Syncrude Fresh Coke (SFC) came straight from the coker whereas it was still hot and dry. Syncrude Aged Coke (SAC) had been slurried with water for transportation and was collected from the ensuing deposit after cooling and weathering.

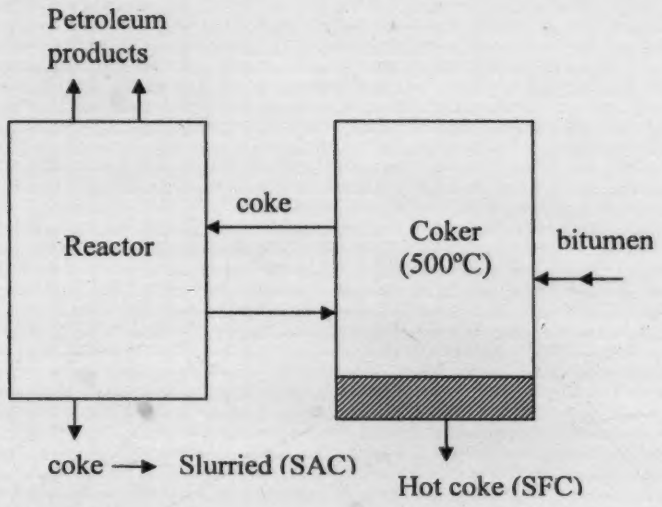

Figure 1-3. Simple schematic of Syncrude fluid coking process

Since Syncrude and Suncor cokes are formed by different processes, they possess different physical characteristics. Syncrude coke consists of fine sandy particles (150 – 200 μm) that are generally round and smooth. The layers that they form resemble that of an onion since they are formed by accreting layers. Suncor coke particles are larger (>1 mm), coarser and the coke surface is more angular (52).

The density of Suncor coke is around 1020 kg/m^3, which is less than that of Syncrude coke (~ 1170 kg/m^3). Suncor coke also contains less moisture than coke from Syncrude (13.1% vs 23%) (52). The higher density of Syncrude coke is likely due to the round shape and uniform size of the particles, which enable them to arrange into a more compact and dense alignment. The higher moisture content in Syncrude coke is probably due to the fluid coking process that it undergoes. Not only does Suncor coke have a low density, it is also hydrophobic. It does not homogeneously mix with water as a slurry, but it usually floats on top of water.

Because coke possesses around the same energy as coal, Syncrude Canada Ltd. burn some of their coke in the coking process, whereas Suncor Energy Inc. uses theirs as a fuel in their utility plant. But since an excess amount of coke is produced each day, most of the coke remains unused. Also, the high sulfur content (6–7%) of petroleum coke from the upgrading of bitumen has discouraged its use as a more general energy source in the open market. Therefore, coke is stored at reclamation sites for possible reuse in the future. At these sites, coke is exposed to precipitation and V has the potential of leaching into the environment.

In the aquatic environment, most surface fresh waters contain less than 3 μg/L of dissolved vanadium (55). Previously, it was concluded that few constituents were released from coke, thus coke should pose no threat to the environment and ground waters (56). However, recent findings reveal that mobilization of certain coke constituents may be occurring under certain conditions. From recent studies done at the University of Saskatchewan, a number of metals leached from SAC reached concentrations that posed possible environmental concern. Metals including cobalt (0.2–35.5 μg/L), copper (0.9–4.9 μg/L), manganese (4.2–559 μg/L), molybdenum (33.6–694 μg/L), vanadium (90.8–2361 μg/L) and zinc (4.8–211 μg/L) were released during aqueous leaching of 25 g of coke with 100 mL of water. Of the mobilized metals, vanadium was observed at the highest concentration (57). Similar experiments have been carried out in our laboratory with simulated environmental settings to assess the speciation of vanadium under different conditions.

1.7.2 Vanadium and *Shewanella putrefaciens* CN32

Not only is vanadium found in coke, it also occurs naturally in the minerals cuprodescloizite, descloizite, mottramite, vanadinite, chileite, patronite and carnotite. In these minerals, V occurs in the oxidation states from 3–5, with the higher oxidation states more toxic and more soluble than the lower ones (58).

Few places in the world contain natural sources of vanadium at high concentrations. One of these is the Colorado Plateau region of the southwestern U.S., which spans about eight states surrounding Colorado and Wyoming. In the 1920s and 30s, major deposits of secondary vanadium ores in the Colorado Plateau region were mined. These ores, which commonly contain high concentrations of uranium, were subsequently reprocessed to support Cold War activities. Tailings and tailings-contaminated materials from mining were disposed of in piles at processing sites throughout the Colorado Plateau region. The alluvial aquifer that underlies the sites is contaminated by seepage from the former mill tailings piles. As a result, groundwater in the Colorado River basin has vanadium concentration ranging from 0.2 to 49.2 μg/L, with the highest levels associated with mining activities (59). High concentrations of vanadium in groundwater correspond to elevated levels of uranium. Consequently, vanadium and uranium have become elements of concern in these areas (60).

Interestingly, the bacterium *S. putrefaciens* CN32 was isolated from the same geographic region where vanadium was mined. *S. putrefaciens* CN32 is a metal-reducing bacterium known to gain energy by using Cr(VI), Mn(IV), Fe(III), U(VI) and Tc(VII) as terminal electron acceptors during respiration (61). This process is termed dissimilatory reduction because the respiring bacteria do not assimilate the metals into integral cellular

components. However, it is not known whether the bacterium could also respire V(V) in the same way. Because the solubility of these metals is greatly diminished at lower oxidation states, bacterial reduction may be useful as a bioremediation technique for environmental effluents contaminated with higher oxidation state of these metals.

In recent studies, it was found that when *Shewanella oneidensis*, a marine isolate, is incubated with V(V), V precipitates as a V(IV) containing solid (62). Shortly after this study, it was found that the strain *S. oneidensis* MR-1, is able to decrease V(V) to V(IV) and this could be used to decrease the toxic impact of V(V) on biological systems (63). Soon thereafter, it was reported that the bacterium *Geobacter metallireducens*, the predominant dissimilatory metal reducer in many freshwater subsurface environments, was also capable of reducing V(V) (64). It was, however, unknown whether the bacteria *S. putrefaciens* CN32, which coexist in its native setting with vanadium, is also able to reduce V(V).

1.8 Research Objectives

The goal of the present work was to develop a method to determine both the quantity and species of vanadium on a routine basis. Ideally, this analytical method should involve an uncomplicated procedure, yet possess sufficient resolution and sensitivity for the determination of vanadium in environmental and biological samples. The analysis time should also be short enough to accommodate many samples on an industrial scale.

Once the method was developed, it must be robust enough to avoid interferences in the matrices of coke pore water and bacterial samples. Some research questions hoped to be answered using the developed technique were:

1. Which species of vanadium and how much of each are present in coke pore water samples collected from Syncrude Canada Ltd.?
2. How does the speciation of V compare amongst Syncrude Fresh Coke, Syncrude Aged Coke and Suncor Delayed Coke?
3. What are the effects of pH and dissolved oxygen on V speciation in these types of coke?
4. Could *S. putrefaciens* CN32 reduce the more toxic V(V) to the less toxic V(IV), or to even lower oxidation states of V?
5. If so, where in the bacteria is the reduction process possibly occurring?

The research on oil sands derived coke will aid oil sands operators to understand how much and which species of V are being mobilized and potentially being made bioavailable. From a water quality perspective, there are concerns about the impact of V contained in this coke on the surrounding environment. Because V(V) is more toxic than V(IV), the speciation of V, rather than the total concentration, must be evaluated to assess the risk of the metal to the environment.

The studies done with V-incubated bacterial cultures can be used to determine if the bacterium is able to reduce V(V) to the less toxic states of the metal, which also happen to be less soluble in water. Once reduced, the insoluble metal could be filtered from the once-contaminated waters. These findings will potentially be useful to industries looking for a way to correct the problem of metal contamination in the environment.

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Chapter 2. Method Development

For the sensitive and simultaneous separation of liquid samples of V(IV) and V(V), a method utilizing HPLC coupled with ICPMS was developed. In order to achieve an optimum separation of the species, several columns and mobile phases were tested with the V standards.

2.1 Instrumentation

2.1.1 HPLC–ICPMS

The HPLC system used for the separation of the V species was the Agilent 1100 Series with an isocratic pump, autosampler, vacuum degasser, thermostated column compartment (Agilent, Germany). The mobile phase was run at 1.0 mL/min for all of the following experiments and the column temperature was maintained at room temperature (~22°C). All samples were injected at a volume of 20 µL.

Vanadium species were detected using the Agilent 7500ce model (Agilent, Japan) ICPMS equipped with shielded torch and octopole collision reaction cell system (Figure 2-1), which was run in the helium mode to remove isobaric interferences. The liquid sample introduction system consisted of a MicroMist nebulizer coupled to a quartz spray chamber. The instrument was tuned on a daily basis using a tuning solution containing Li, Mg, Y, Ce, Tl and Co at 1 ng/mL each. The tuning solution was carried to the nebulizer pump at 0.3 mL/min using a Gilson peristaltic pump.

Page 29 has been removed due to copyright restrictions.

The information removed was Fig 2-1. Inductively coupled plasma mass spectrometer

Source: Agilent Technologies website. http://www.chem.agilent.com/Scripts/PHome.asp

Table 2-1. ICPMS operating conditions

Parameter	Setting
RF power	1550 W
Carrier gas flow rate (Ar)	0.96 L/min
Make up gas flow rate (Ar)	0.27 L/min
Octopole RF	174 V
Octopole bias	-18.2 V
Octopole gas flow rate (He)	2.5 mL/min
QP bias	-16 V
Spray chamber temp	2.0 °C
Sampler and skimmer cones	Platinum
Sampling depth	8.2 mm

2.1.2 ESI-MS

An Applied Biosystems/MDS Sciex QSTAR Pulsar i mass spectrometer (Concord, ON, Canada) equipped with an ionspray ionization source was used to characterize the V-EDTA standards. The quadrupoles were operated in the RF mode to allow total transmission of the ions as shown in Figure 2-2. A time-of-flight (TOF) analyzer was used to maximize resolution.

Page 31 has been removed due to copyright restrictions.

The information removed was Fig 2-2. Hybrid quadrupole TOF system

Source: Applied Biosystems. AB/MDS SCIEX QSTAR Training Course slides.

USA) was used for data acquisition and analysis. IgorPro software (Wavemetrics, Lake Oswego, OR, USA) was used to plot the mass spectra.

2.2 Preparation of V Standards

Deionized distilled water (DIW) was used to prepare all solutions. All chemicals and solvents were of HPLC grade. Stock standard solution of 1.0 g/L V(IV) was prepared by dissolving 0.50 g of $VOSO_4 \cdot 5H_2O$ (Riedel de Haën) in 100 mL of DIW. V(V) standard (1.0 g/L) was prepared by dissolving 0.23 g of ammonium metavanadate (Fluka) in 2 mL concentrated optima HNO_3 (Fisher), then diluting the solution to 100 mL with DIW.

The V-EDTA stock solutions were made by adding 0.215 g (1.5 times equimolar proportions) of EDTA acid (Sigma) to 25 mL of each V stock. The mixtures were heated gently and sonicated for 10 min to achieve maximum dissolution of EDTA. They were then vortexed and allowed to stand for 15 min for complete chelation. During this time, V(IV) stock solution changed from blue to purple whereas V(V) solution stayed yellow. The pH of each V-EDTA stock solution was adjusted to 6.0 using HNO_3 and NH_4OH. These stock solutions were then diluted to 50 mL with DIW and sonicated. Fresh standard samples were diluted from these stocks and mixed daily for analysis. Solution mixtures of $[VOY]^{2-}$ and $[VO_2Y]^{3-}$ (10 µg/L each) were used for all the method development experiments.

The $[VY \cdot H_2O]^-$ standard was synthesized as $Na[V(EDTA)(H_2O)] \cdot 3H_2O$ by modifying a procedure outlined by Shimoi et al. (1). Instead of using a cation exchange resin to obtain the sodium salt, $Na[V(EDTA)(H_2O)] \cdot 3H_2O$ was directly synthesized from Na_4EDTA. Excess Na_4EDTA (2.0 g) was dissolved in 10 mL of DIW and 803 mg of

VCl_3 was weighed in a dark glovebox since it is sensitive to O_2 and light. The EDTA solution was mixed with VCl_3 under Schlenk lines and the mixture was allowed to react for 25 min. Ethanol was then added to precipitate a dark brown solid. The product was filtered under vacuum and washed with ether. The dried weight of the product was 1.864 g and the percent yield was 85%. The $[VY{\cdot}H_2O]^-$ complex solution (100 mg/L) was made by dissolving 42.6 mg of the synthesized product in 50 mL of DIW. This yellow stock solution was stable for only one month and the solid standard also lost stability with time. The $[VY{\cdot}H_2O]^-$ standard was not used for any of the method development experiments because it did not become a species of interest until the bacteria samples were analyzed.

2.3 ESI-MS data of V-EDTA Standards

To confirm that the EDTA was successfully complexed to vanadium, each standard was prepared at 100 μg/L and analyzed on the QStar mass spectrometer. The theoretical m/z for $[VY{\cdot}H_2O]^-$, $[VOY]^{2-}$ and $[VO_2Y]^{3-}$ are 357.17, 177.62 and 123.75, respectively.

The theoretical structure of $[VY{\cdot}H_2O]^-$ is a seven-coordinated molecule with a water molecule completing its seventh coordination. The structure has been described as a capped trigonal prism (1) as shown in Figure 2-3.

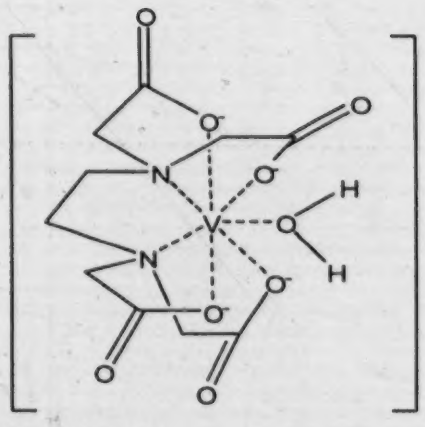

Figure 2-3. Structure of $[VY{\cdot}H_2O]^-$

The synthesized $[VY{\cdot}H_2O]^-$ was prepared at 400 µg/L in DIW and diluted twice with MeOH in a glass vial. The standard was analyzed on the mass spectrometer in the negative mode under ionspray ionization.

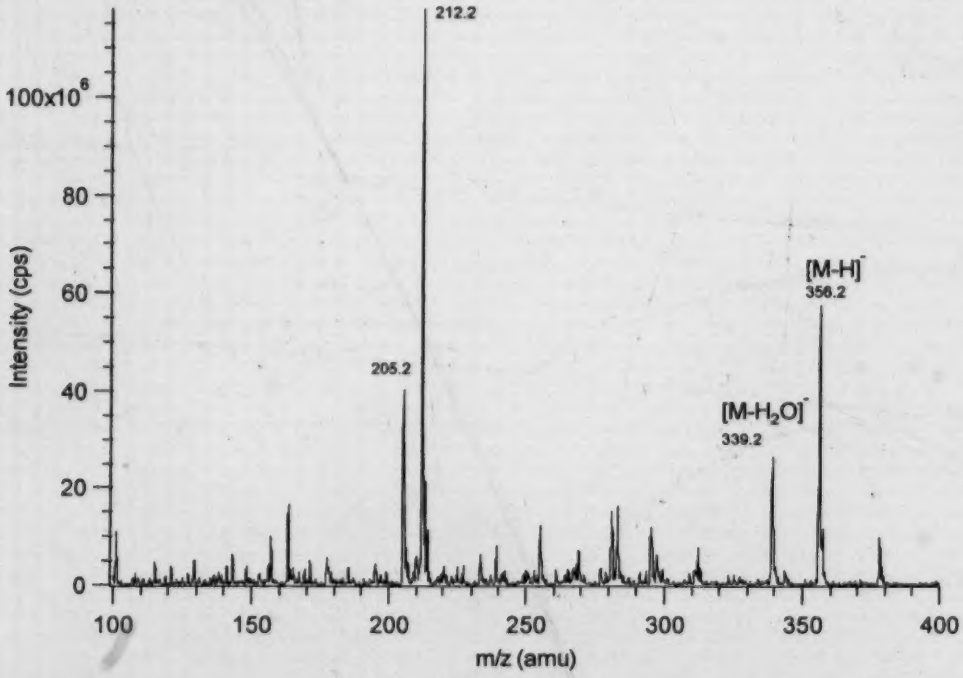

Figure 2-4. Mass spectrum of 200 µg/L $[VY{\cdot}H_2O]^-$ in MeOH. IS = -4500 V, DP = -50 V, CE = -10 V, GS1 = 15, CUR = 25, flow rate = 40 µL/min

From Figure 2-4, two peaks at m/z 356.2 and 339.2 are clearly observed. The former corresponds to the deprotonated parent ion and the latter is due to the loss of the water ligand. To make sure that these two peaks are not from the background, a blank of 50% MeOH made up in DIW was analyzed (Figure 2-5).

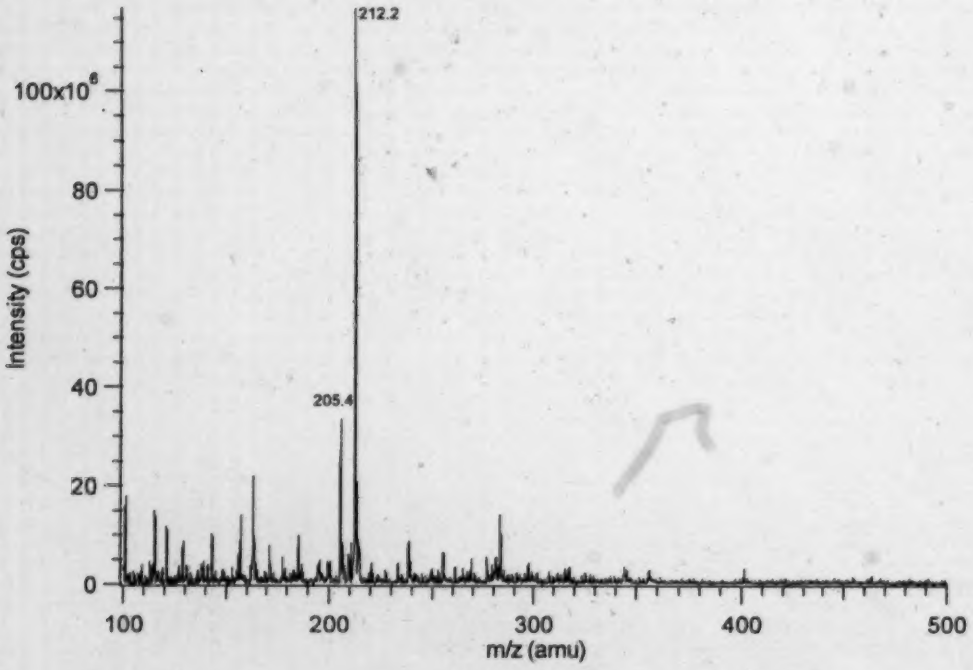

Figure 2-5. Mass spectrum of 50% MeOH blank. IS = -4500 V, DP = -50 V, CE = -10 V, GS1 = 15, flow rate = 40 μL/min

The absence of the signature parent peaks in the blank (Figure 2-5) indicates that the standard was successfully synthesized. The identity of the $[VY{\cdot}H_2O]^-$ complex is further supported by the HPLC-ICPMS data shown in Figure 2-33.

The structure for the $[VOY]^{2-}$ is likely a five-coordinate species (2) with the central V only coordinating with oxygen (Figure 2-6) since a seven-coordinate structure has never been observed with V(IV) (3).

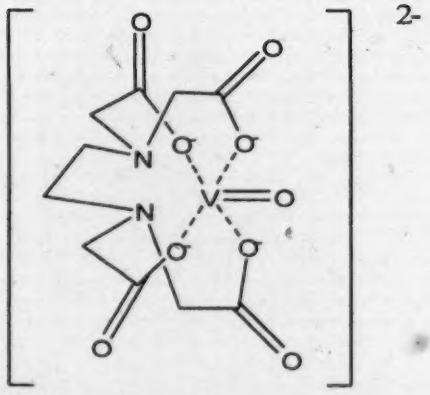

Figure 2-6. Probable structure of $[VOY]^{2-}$

The EDTA complex of V(IV) was made up with methanol and DIW in a 1:1 volume ratio in a glass vial. It was later analyzed on the ESI-MS via ionspray. A mass spectrum (Figure 2-7) was obtained in the negative mode.

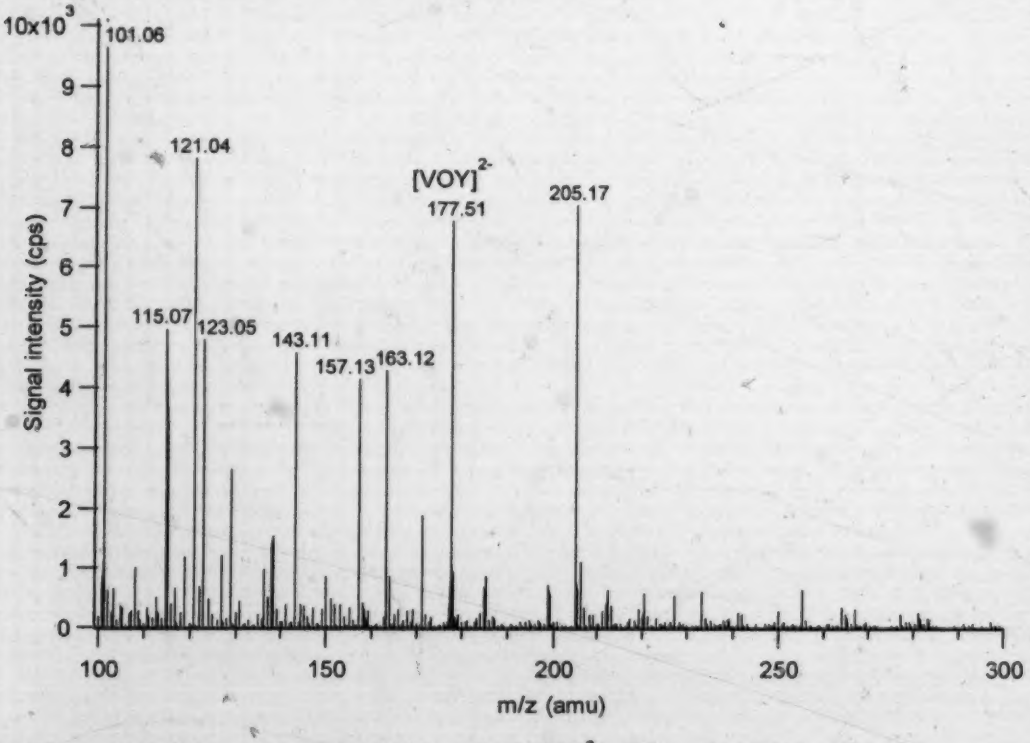

Figure 2-7. Mass spectrum of 100 µg/L $[VOY]^{2-}$. DP = -20 V, FP = -75 V, IS = -4000 V, GS1 = 30, CUR = 25

Because the V=O bond is strong, it does not dissociate, resulting in the observed parent peak at m/z 177.51. In order to confirm this ion is not from the background, a blank solution consisting of 50% MeOH was analyzed as shown in Figure 2-8.

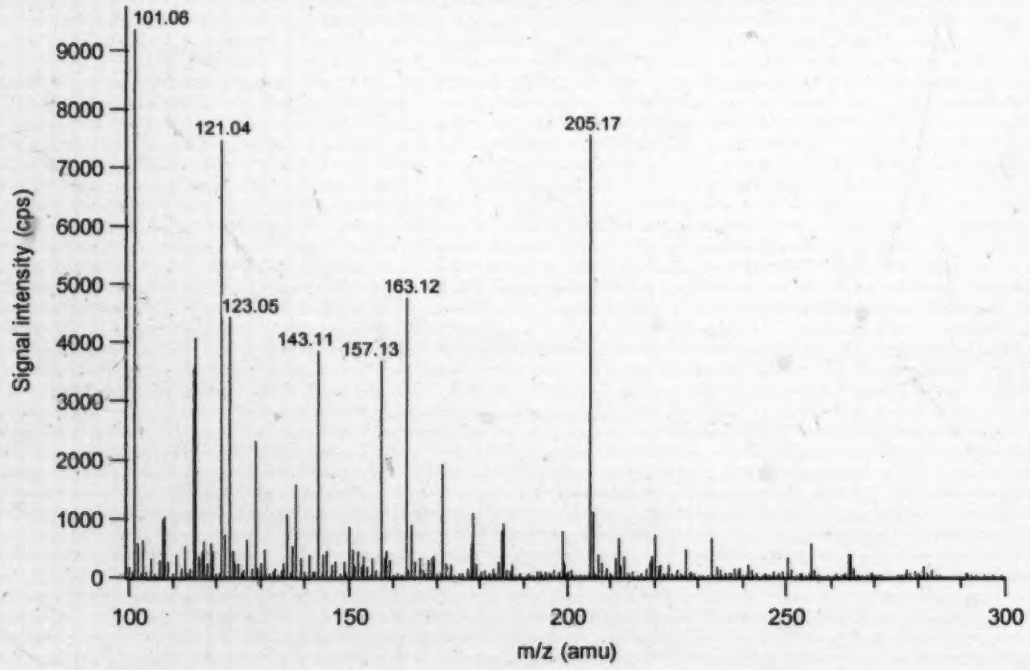

Figure 2-8. Mass spectrum of 1:1 MeOH:DIW blank. DP = -20 V, FP = -75 V, IS = -4000 V, GS1 = 30, CUR = 25

The absence of the peak at m/z 177.51 confirms that this peak is indeed due to the $[VOY]^{2-}$ standard.

The $[VO_2Y]^{3-}$ complex is likely a six-coordinate complex possessing an octahedral geometry with a cis-dioxo group (4), its structure is shown below:

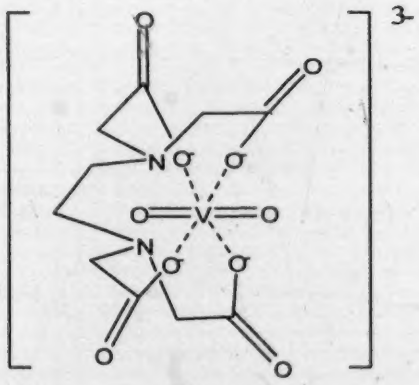

Figure 2-9. Probable structure of $[VO_2Y]^{3-}$

To verify the identity of the $[VO_2Y]^{3-}$ standard, 100 µg/L $[VO_2Y]^{3-}$ prepared in MeOH was run in the negative mode. The mass spectrum in Figure 2-10 was obtained.

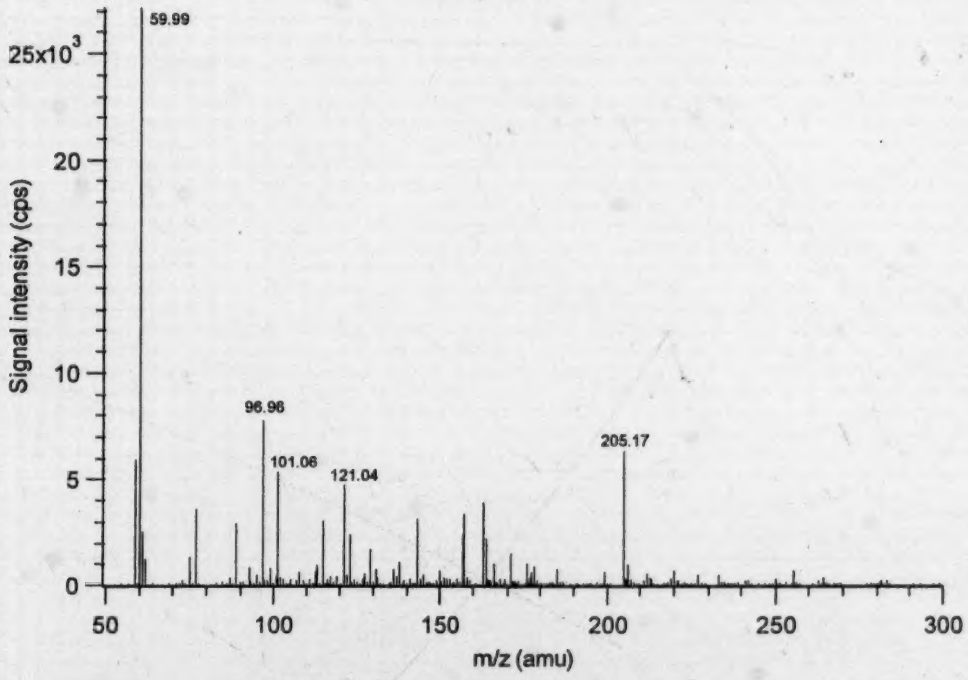

Figure 2-10. Mass spectrum of 100 µg/L $[VO_2Y]^{3-}$ in MeOH. DP = -20 V, FP = -75 V, IS = -4000V, GS1 = 30

Examining the spectrum above, the $[VO_2Y]^{3-}$ peak at m/z 123.75 was not present. This probably indicates that the $[VO_2Y]^{3-}$ complex does not have the same stability as the $[VOY]^{2-}$ complex in the mass spectrometer and is probably fragmented in source. If the $[VO_2Y]^{3-}$ complex did in fact dissociate, it would fragment to give the inorganic VO_2^+ ion and the $EDTA^{4-}$ ions. To detect the VO_2^+ fragment, the $[VO_2Y]^{3-}$ standard was analyzed in the positive mode. Isopropanol was chosen as the solvent because it usually affords the best sensitivity for detecting the small inorganic V ions as found by experience.

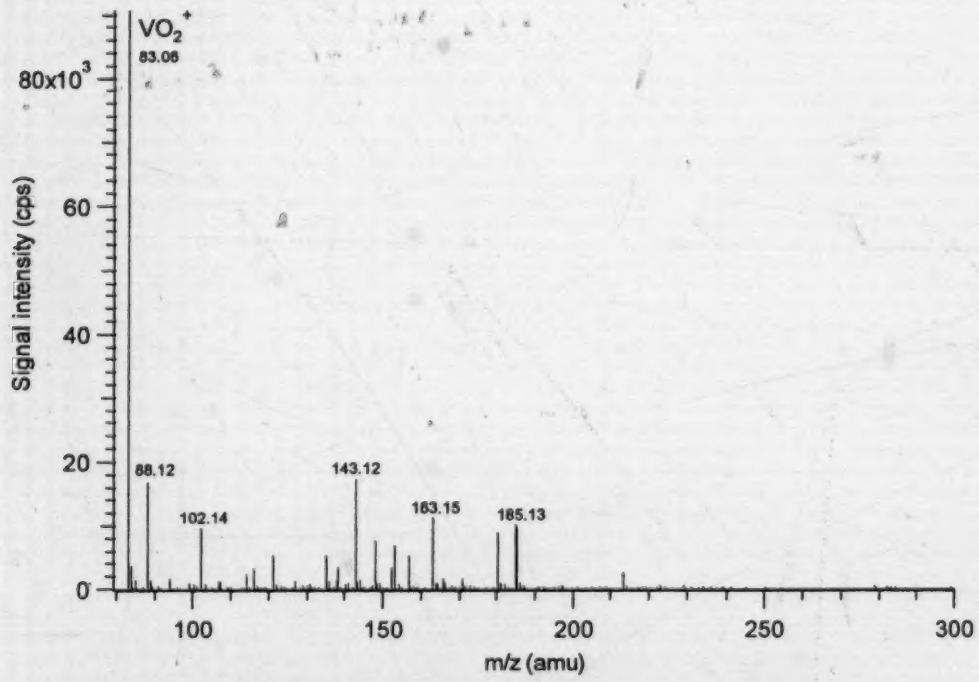

Figure 2-11. Mass spectrum of 100 µg/L $[VO_2Y]^{3-}$ in iPrOH. DP = 20 V, FP = 75 V, IS = 4000 V, GS1 = 30

An intense peak at m/z 83.06 is observed in Figure 2-11. The species responsible for this peak is probably the inorganic V(V) ion VO_2^+, which would indicate the

dissociation of the complex. However, when the blank solution was analyzed, the same peak was present (Figure 2-12), but it was less intense.

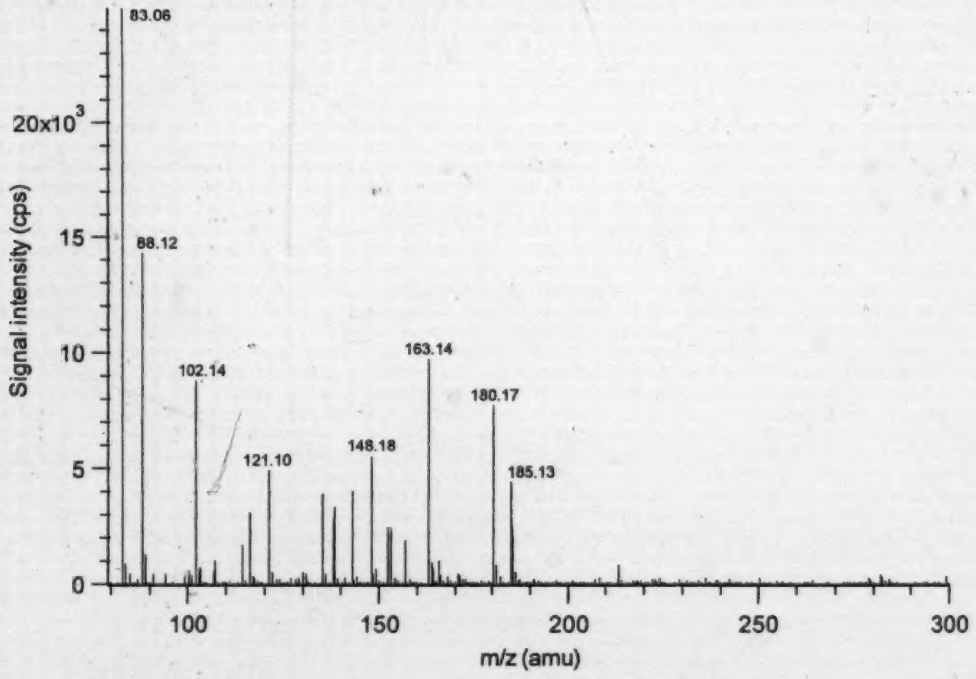

Figure 2-12. Mass spectrum of 50% iPrOH blank. DP = 20 V, FP = 75 V, IS = 4000 V, GS1 = 30

To confirm that the peak at m/z 83.05 is indeed due to VO_2^+ and not the background, a direct injection from the HPLC of 200 µg/L $[VO_2Y]^{3-}$ was done without using a column. The mobile phase used to elute the compound was 50% iPrOH. The column flow rate was set at 10 µL/min, injection volume was 3 µL, draw speed was 4 µL/min and ejection speed was 10 µL/min.

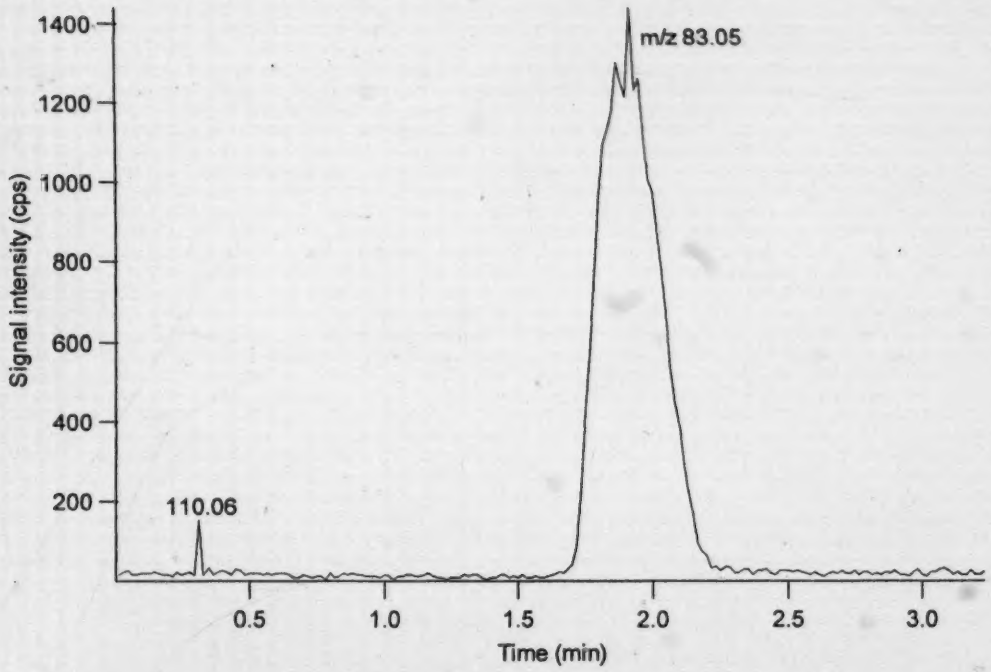

Figure 2-13. Total ion chromatogram of 100μg/L $[VO_2Y]^{3-}$/iPrOH. DP = 20 V, FP = 75 V, IS = 3000 V, GS1 = 30

From the chromatogram in Figure 2-13, we can see that the peak at 1.9 min is due to the analyte $[VO_2Y]^{3-}$. When this peak was selected, it afforded the spectrum shown in Figure 2-14.

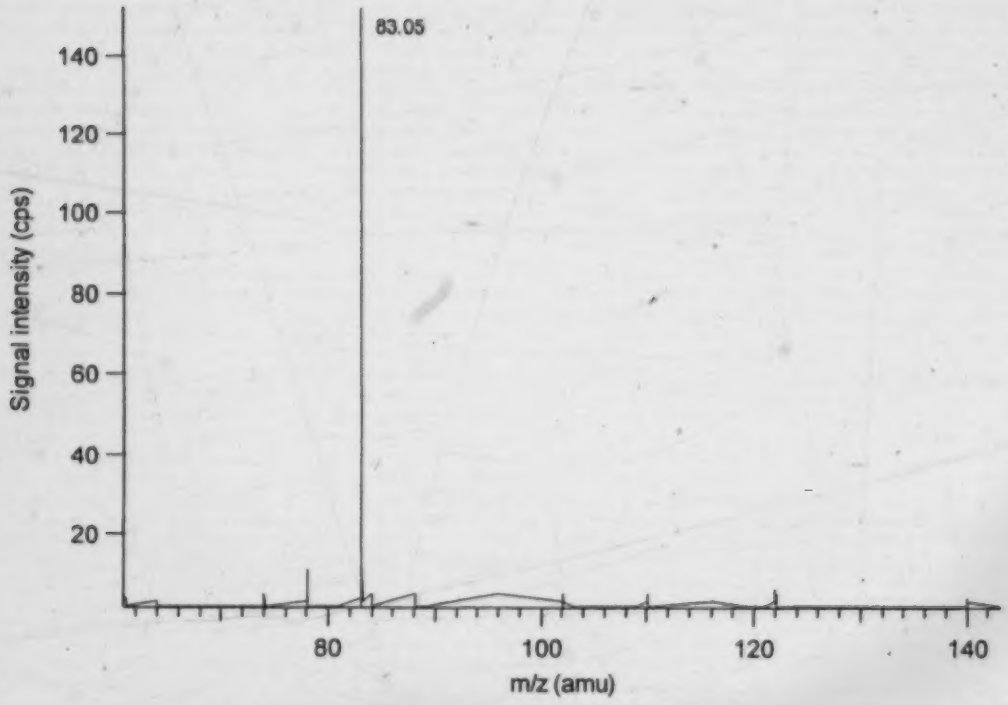

Figure 2-14. Mass spectrum of the selected peak at 1.9 min.

When the background spectrum at 1.0 min from Figure 2-13 was chosen, it did not give any ion peaks in the mass spectrum. Thus we can conclude that the peak at m/z 83.0 is indeed due to the fragmented VO_2^+ ion that has dissociated from $[VO_2Y]^{3-}$ complex, and not from the background.

From the data and discussion above, some spectrometric behavior of the $[VOY]^{2-}$ and $[VO_2Y]^{3-}$ complex can be deduced. The former showed up as an intact complex and its signature peak was observed at m/z 177.51. In the latter, the EDTA ligand dissociated from the complex and only the inorganic VO_2^+ ion at m/z 83.0 was observed. $[VY{\cdot}H_2O]^-$ has an intermediate stability and only the H_2O ligand dissociated from the complex.

Due to the lack of color change when the V(V) ion is complexed and the absence of the $[VO_2Y]^{3-}$ peak in the mass spectrum, there might still be some suspicion as to

whether this complex was indeed formed. In fact, $[VO_2Y]^{3-}$ is the most stable species of V because V cannot oxidize to higher oxidation states above (V). In addition, the $[VO_2Y]^{3-}$ peak was observed to be stable after a year of making up the standard, whereas $[VOH_2O]^-$ and $[VO_2Y]^{2-}$ were only stable for one month and six months, repectively.

2.4 Preliminary Studies on Method Development

The solution chemistry of the vanadium ions is very complicated. At pH 2 where the V(IV) ion is stable, the solution appears blue and V(IV) is believe to exist as VO^{2+}. When the pH increases to around 5, the solution turns brown and the ion responsible for this color is $V_{18}O_{42}^{12-}$ (5). When the solution becomes more basic, V(IV) is oxidized to V(V) or VO_4^{3-}. Above pH 13, V(V) is mainly VO_4^{3-}. In the pH range 2– 6, V(V) appears as the orange decavanadate ion, which can exist in the forms of $V_{10}O_{28}^{6-}$, $V_{10}O_{27}(OH)^{5-}$, $V_{10}O_{26}(OH)_2^{4-}$, $V_{10}O_{25}(OH)_3^{3-}$, $V_{10}O_{24}(OH)_4^{2-}$ and eventually VO_2^+, as the solution becomes more acidic (3). At low pH, V(IV) and V(V) exist as VO^{2+} and VO_2^+, respectively. Thus they should theoretically be separated on a cation exchange column with the elution order of V(V), then V(IV). According to previously reported studies, V(IV) and V(V) should be able to separate at pH 1.6 on a cation exchange column (6). To test this, 20 μg/L of inorganic V(IV) and V(V) ions were each made up in DIW adjusted to pH 2 with HNO_3. Each standard was injected separately and in combination onto the 2 cm long Sep-Pak Plus CM cartridge (Waters, USA) using a 0.16% HNO_3 solution at pH 1.65 as the eluent. The cartridge is a silica based hydrophilic weak cation exchanger. Its stationary phase is a carboxyl-methyl functional group.

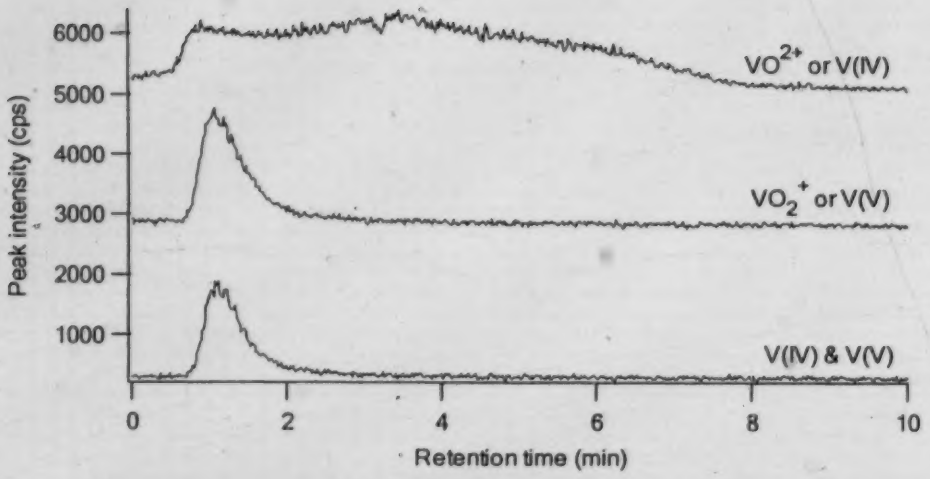

Figure 2-15. Retention of uncomplexed V on cation exchange cartridge. Eluent, DIW adjusted to pH 2 with HNO_3

According to Figure 2-15, VO^{2+}, the more cationic species, is retained on the column much longer than VO_2^+. However, when the two ions are combined, only the V(V) peak appeared in the chromatogram. This could be due to the oxidation of V(IV) to V(V) by air and/or the oxidizing HNO_3. It is common for the labile V(IV) to oxidize to V(V) under oxic conditions.

Thus in order for the V(IV) and V(V) species to remain stable on column, they need to be fixated with a ligand.

2.5 Reversed Phase Ion Pair Chromatography

When complexed with EDTA, both species of V have relatively high formation and stability constants (7). In fact, the $[VOY]^{2-}$ and $[VO_2Y]^{3-}$ standards made up in the laboratory were stable for 7 months and 1 year, respectively.

In addition, $[VOY]^{2-}$ and $[VO_2Y]^{3-}$ differ by a charge of 1. This difference makes the separation possible using ion pair chromatography on a reversed phase Supelcosil

LC-8 column (25 cm x 4.6 mm x 5 μm, Supelco, Oakville, ON). The two ions interact with the ion pairing agent TBA differently (8) and are retained to different extents on the column. Jen and Yang (8) used liquid chromatography with UV detection. The eluent they employed was 12% ACN, 50 mM TBA and 2 mM EDTA at pH 6. Due to the nature of the ICPMS, it can only withstand up to 5% of organic solvent. Thus 4% ACN along with 50 mM TBA and 2 mM EDTA at pH 6 was tested as the eluent. The chromatogram in Figure 2-16 was obtained when running a mixture of the V species with each made up at 5 μg/L.

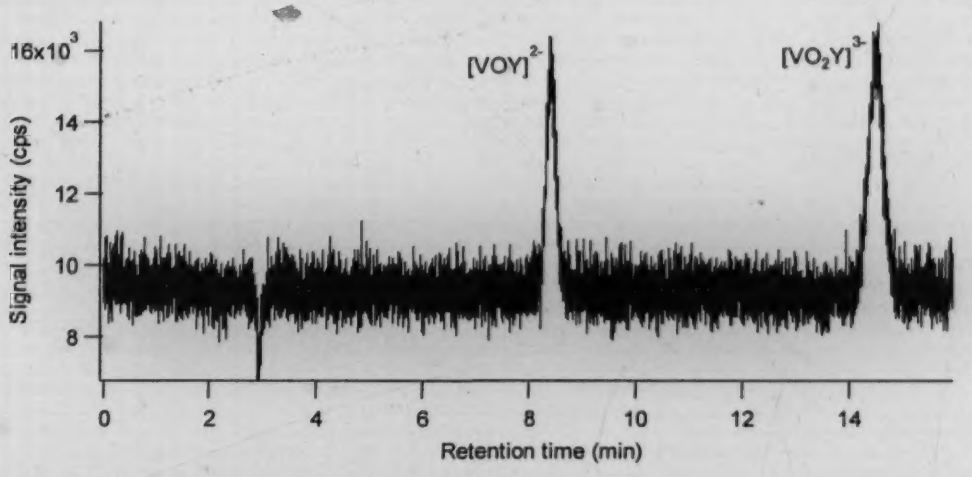

Figure 2-16. Reversed phase speciation on a C8 column. Eluent consists of 4% ACN, 50 mM TBA, 2 mM EDTA at pH 6.0

Because $[VO_2Y]^{3-}$ has a higher charge than $[VOY]^{2-}$, it has a stronger interaction with TBA and is more retained on the column. The resolution was quite good, but the analysis time was too long.

In the attempt to shorten the analysis time, tetraethyl ammonium (TEA) was used since the shorter carbon chain should interact to a lesser extent with V-EDTA and the C8 stationary phase. The chromatogram in Figure 2-17 was obtained using 4% ACN, 30

mM TEA, 2 mM EDTA and 3 mM ammonia formate at pH 6 as the eluent. Ammonia formate was used as the buffer to stabilize the pH of the eluent. Each V-EDTA standard was made up at 20 µg/L.

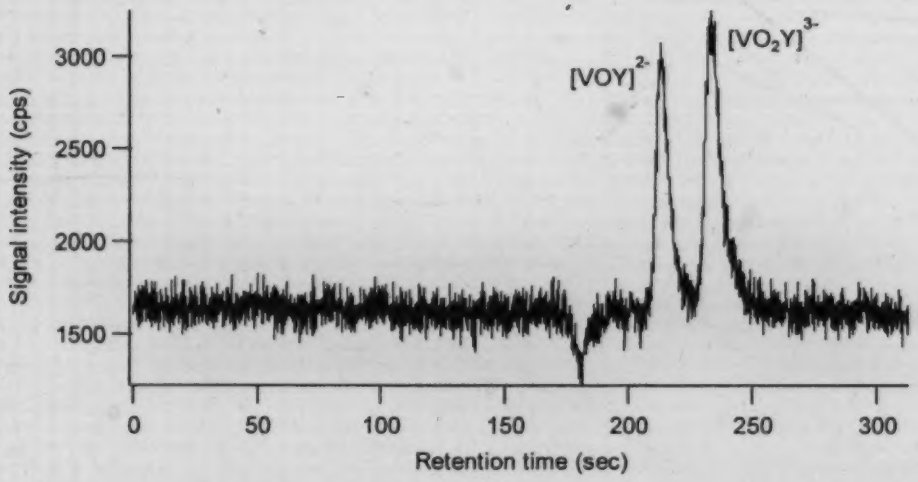

Figure 2-17. Reversed phase speciation with TEA as the ion pairing agent. Eluent consists of 4% ACN, 30 mM TEA, 2 mM EDTA and 3 mM ammonia formate at pH 6.0

The less bulky ion pair agent did release the V-EDTA sooner from the stationary phase compared to TBA, but the two complexes now also have similar retentions on the column with this weaker interaction, causing the two peaks to be barely baseline resolved. It would be better for future analysis if the two species were better separated in case there is an interfering ion with a similar retention time.

In real samples, V will not be naturally bound to EDTA. If the inorganic V can be complexed with EDTA from the mobile phase on line, sample pretreatment with EDTA could be avoided. To test this, a mixture of inorganic V(IV) and V(V) were analyzed on the C8 column with 4% ACN, 40 mM TEA, 4 mM EDTA and 3 mM ammonia formate at pH 6.2. The resulting chromatogram is shown in Figure 2-18.

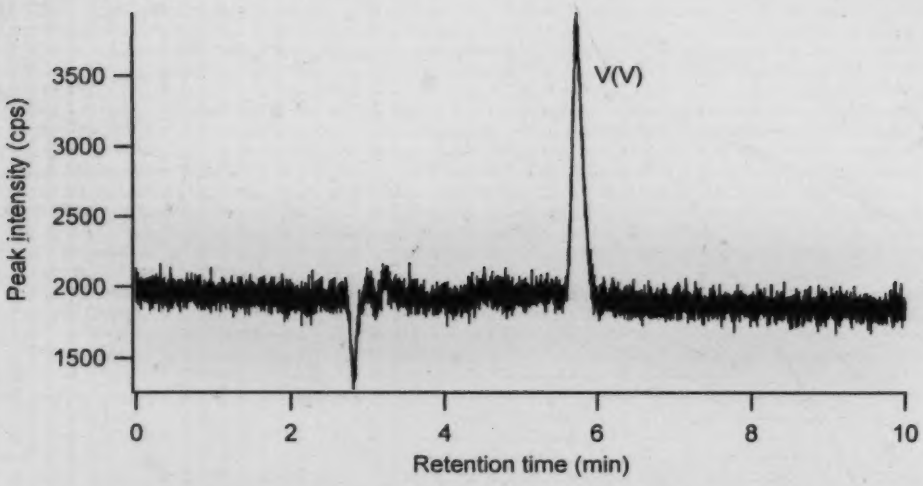

Figure 2-18. Mix of inorganic V(IV) and V(V) (20 μg/L each) without pretreatment with EDTA. Eluent consists of 4% ACN, 40 mM TEA, 4 mM EDTA and 3 mM ammonia formate at pH 6.2

Only the V(V) peak appeared in Figure 2-18 since V(IV) was oxidized. Thus it is essential that EDTA be added to the sample before they are to be analyzed.

To improve resolution, a cationic stationary phase can be used such that the retention would not depend on the ion pairing agent. By having the vanadium ions solely interact with the stationary phase, we would have one less component to optimize later in the study.

2.6 Development of SAX Method

In the effort to develop a novel method for speciating V(IV) and V(V) with sufficient resolution and short analysis time, a PRPX100 strong anion exchange (SAX) column (50 mm x 4.1 mm i.d. x 3 μm, Hamilton, Reno, Nevada) was used. The LC eluent was prepared from ACN (EMD), EDTA acid (Aldrich) and NH_4HCO_3 (Sigma). NH_4HCO_3 was chosen since it has a buffer range between 5.4 and 7.4, which brackets the desired pH 6. Nitric acid (1 M) and NH_4OH (3 M) were used to adjust the pH. Even

though it is an oxidizing agent, nitric acid was chosen since it readily decomposes to NO_2 in the ICPMS. All eluents were filtered through a 0.45 μm Nalgene filter and degassed ultrasonically. Fresh working solutions of the eluent were made up daily.

After fine tuning each component, the optimum eluent was determined to consist of 3% ACN, 2 mM EDTA and 80 mM NH_4HCO_3 at pH 6.0. This condition was chosen to achieve the shortest analysis time with good resolution and high sensitivity. A chromatogram of the two species (10 μg/L each) is shown below.

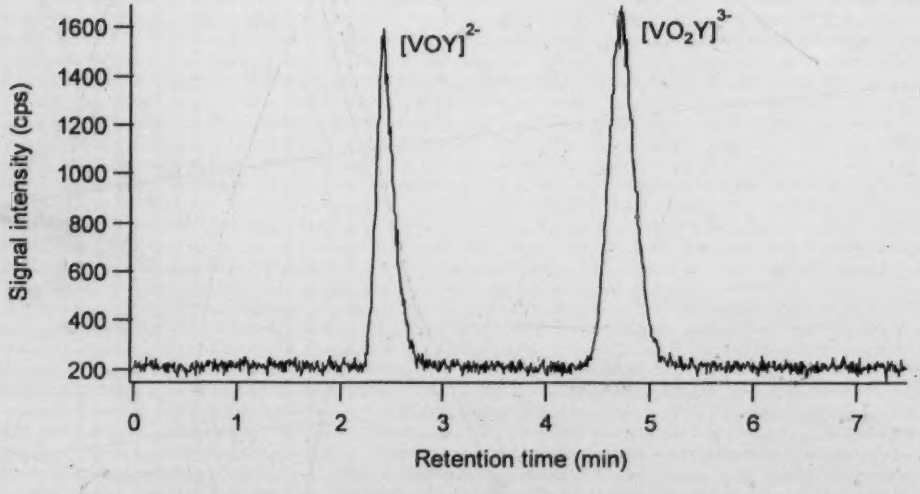

Figure 2-19. Optimum separation of $[VOY]^{2-}$ and $[VO_2Y]^{3-}$ on the SAX column. Eluent, 3% ACN, 2 mM EDTA and 80 mM NH_4HCO_3 at pH 6.0. Dead time of the column was approximately 0.6 min

The following discussions show how pH, organic modifier, NH_4HCO_3 and EDTA were each optimized to compromise between resolution, analysis time, reproducible peak areas and sensitivity. The EDTA complexes of V(IV) and V(V) were used for all the following experiments.

2.6.1 Effect of pH

A very important factor in the separation of V-EDTA complexes is pH since the speciation of V largely depends on it. It is known that below pH 5, $[VO_2Y]^{3-}$ is protonated to form $[VO_2HY]^{2-}$, $[VO_2H_2Y]^{-}$ and $[VO_2H_3Y]$. Above pH 10, vanadium species condense (7). In this study, pH 5, 6 and 7 were investigated.

Table 2-2. Effect of pH on retention time (t_r) and peak area (PA)

pH	t_r V(IV) (min)	t_r V(V) (min)	PA V(IV)	PA V(V)	Resolution
5.0	2.26 ± 0.02[a]	4.35 ± 0.07	45000 ± 1000	47500 ± 300	4.7
6.0	2.14 ± 0.02	3.97 ± 0.03	41300 ± 900	42100 ± 600	4.2

[a]Error is calculated based on the standard deviation of triplicate injections.

When the mobile phase was at pH ≥ 7, V(IV) was completely oxidized to V(V) when a mixture of the two standards was analyzed. According to Table 2-2, samples analyzed at pH 5 actually had slightly better (1.1 times) resolution and sensitivity than at pH 6, though analysis time was slightly longer (by 0.38 min). However, since pH 6 is closer to those of the environmental and biological samples, the pH was maintained at 6 for the remainder of the study.

2.6.2 Effect of organic modifier

Organic modifiers were added in the attempt to shorten analysis time and improve resolution. Initially, two mobile phases were made. One contained 4% methanol, 70 mM NH_4HCO_3 and 5 mM EDTA at pH 6. The other contained the same components except the organic modifier used was 4% ACN. It was found that the latter gave a shorter analysis time (11.3 min vs 15.0 min) and a better recovery of vanadium species from the column (97% vs 91%). Recovery was determined by the total V peak area obtained from

a direct injection of the standard without the column and the added peak areas of the V species from running the standard on the column. Thus ACN was chosen as the organic modifier. To optimize the concentration of ACN, mobile phases consisting of 1, 2, 3 or 4 percent ACN with 10 mM EDTA and 80 mM NH_4HCO_3 at pH 6.0 were tested. The percentage was not increased above 4% since the plasma of the ICPMS can only withstand up to 5% organic solvent. According to Figure 2-20, retention of $[VO_2Y]^{3-}$ was hardly affected by the amount of ACN present whereas $[VOY]^{2-}$ was eluted slightly faster with a higher ACN content. More importantly, resolution increased with increasing concentration of ACN (Figure 2-21). However, an increasing amount of ACN seems to decrease sensitivity until sensitivity plateaus at 3% (Figure 2-22). The error bars in Section 2.6 represent reproducibility of the instrument with triplicate injections. The legends V4E and V5E refer to the EDTA complexes of V(IV) and V(V), respectively. Note that some of the error bars appear smaller than the points themselves.

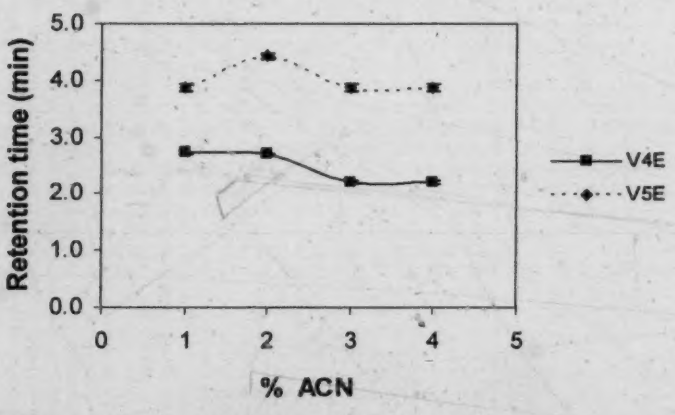

Figure 2-20. Effect of ACN concentration on retention time. Eluent, x % ACN, 10 mM EDTA and 80 mM NH_4HCO_3 at pH 6. Data were average of triplicate analyses. Error bars representing $\pm$ one standard deviation were smaller than the size of the symbols

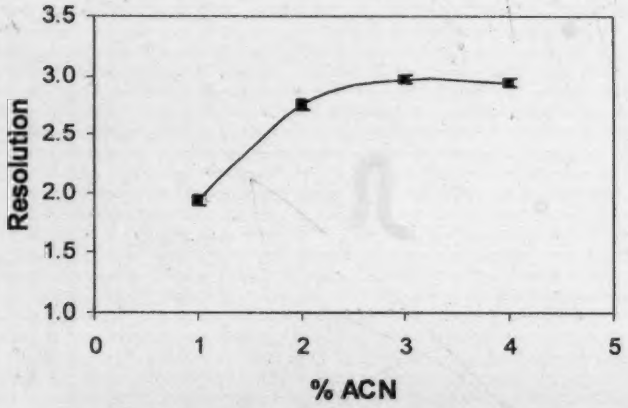

Figure 2-21. Effect of ACN concentration on resolution. Eluent, x % ACN, 10 mM EDTA and 80 mM NH_4HCO_3 at pH 6. Data were average of triplicate analyses. Error bars representing ± one standard deviation were smaller than the size of the symbols

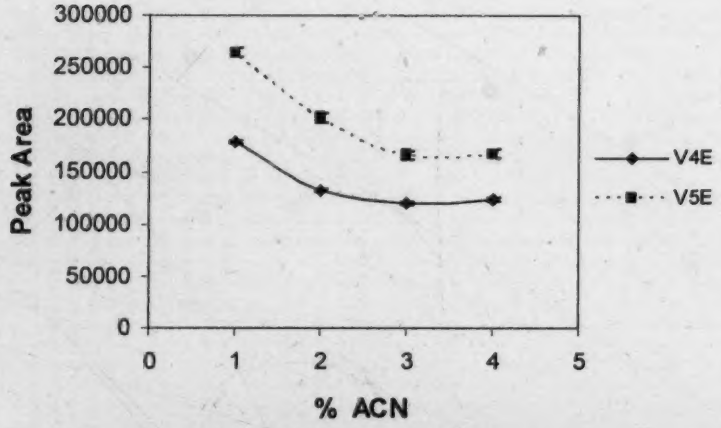

Figure 2-22. Effect of ACN concentration on peak areas. Eluent, x % ACN, 10 mM EDTA and 80 mM NH_4HCO_3 at pH 6. Data were average of triplicate analyses. Error bars representing ± one standard deviation were smaller than the size of the symbols

Although 3% ACN results in a lower sensitivity than with lower concentrations of ACN, it affords the best resolution. Therefore it is chosen for future analysis.

2.6.3 Effect of NH_4HCO_3

Ammonium bicarbonate was chosen as the buffer since it has a buffering capacity compatible with the chosen pH. Besides resisting pH changes, addition of NH_4HCO_3 increases the ionic strength of the eluent such that the analysis time can be shortened. The experiments were performed with 60, 70, 80 or 90 mM NH_4HCO_3, 3% ACN and 10 mM EDTA at pH 6.0.

As expected, increasing [NH_4HCO_3] decreased the retention time of V as seen in Figure 2-23, especially for $[VO_2Y]^{3-}$ due to its higher charge. Increasing the concentration from 60 mM to 90 mM NH_4HCO_3 did not have any significant impact on sensitivity (Figure 2-24).

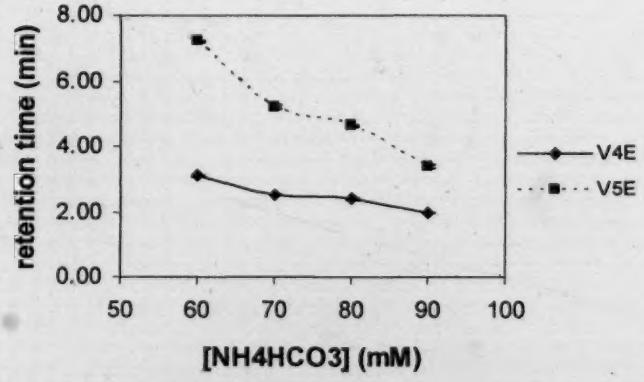

Figure 2-23. Effect of [NH_4HCO_3] on retention time. Eluent, x mM NH_4HCO_3, 3% ACN and 10 mM EDTA at pH 6.0. Data were average of triplicate analyses. Error bars representing ± one standard deviation were smaller than the size of the symbols

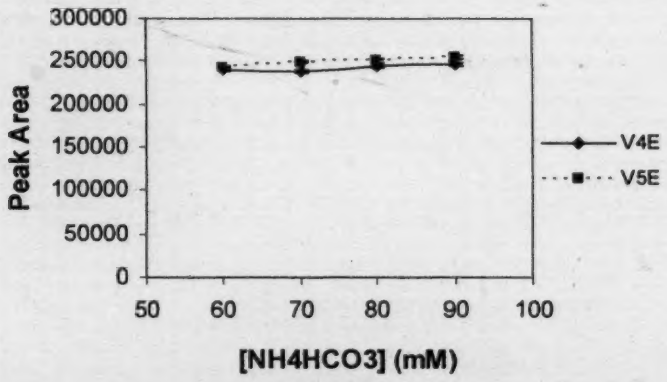

Figure 2-24. Effect of $[NH_4HCO_3]$ on peak area. Eluent, x mM NH_4HCO_3, 3% ACN and 10 mM EDTA at pH 6.0. Data were average of triplicate analyses. Error bars representing $\pm$ one standard deviation were smaller than the size of the symbols

Even though 60 mM NH_4HCO_3 gave the best resolution, the analysis time was too long (8 min) for routine analysis and the sensitivity was lower than the rest. A concentration of 80 mM affords an analysis time under 5 min with good resolution, thus it was chosen.

2.6.4 Effect of EDTA

EDTA is an essential component of the mobile phase since it stabilizes the V(V) complex (8). It is known that although the $[VO_2Y]^{3-}$ complex has a high formation constant (log K=15.55) (7) and the standard is stable for up to 7 months of storage, it is not stable in the elution system until EDTA is added to the eluent. Without EDTA in the elution system, the $[VO_2Y]^{3-}$ peak is significantly broadened and decreased (Figure 2-25). On the other hand, the $[VOY]^{2-}$ signal appears stable (log K=18.8) (7) in the absence of EDTA in the eluent (Figure 2-26).

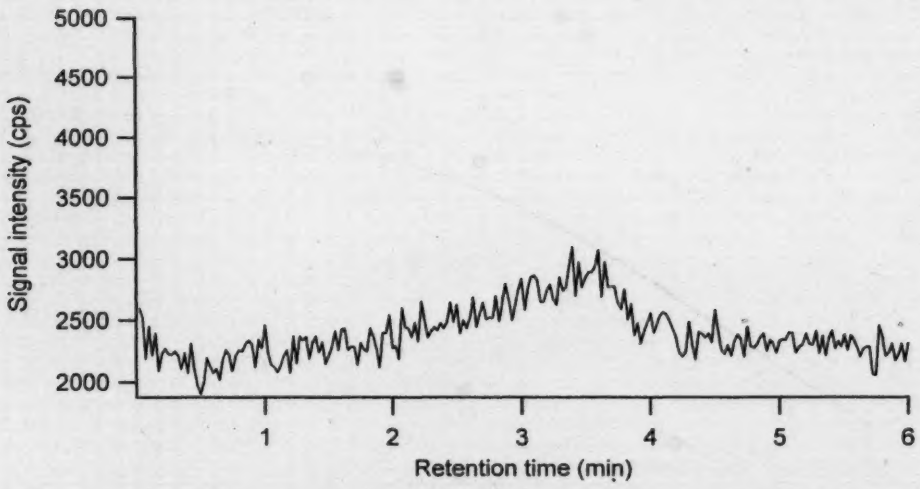

Figure 2-25. $[VO_2Y]^{3-}$ (25 µg/L) analyzed without EDTA in the eluent. Eluent, 3% ACN and 80 mM NH_4HCO_3 at pH 6.0

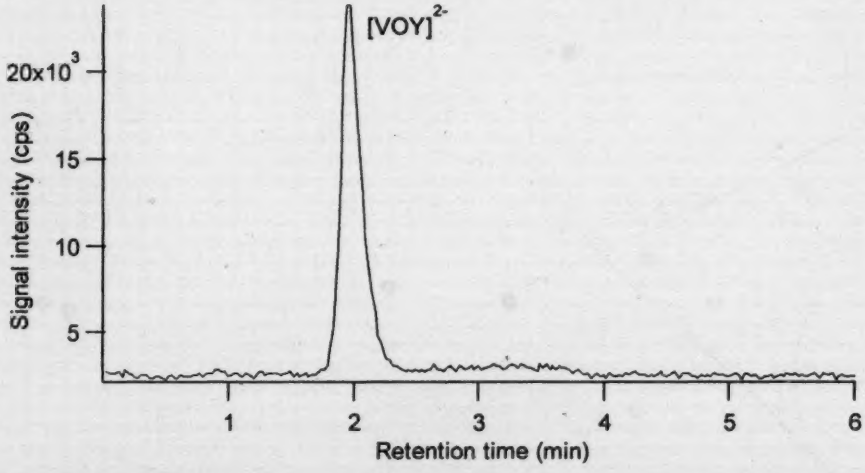

Figure 2-26. Mix of $[VOY]^{2-}$ and $[VO_2Y]^{3-}$ (25 µg/L) analyzed without EDTA in the eluent. Eluent, 3% ACN and 80 mM NH_4HCO_3 at pH 6.0

The eight tested mobile phases contained 0, 0.2, 0.4, 0.6, 2, 5, 10 or 15 mM EDTA. The other common components are 3% ACN and 80 mM NH_4HCO_3 at pH 6.0. The dependence of retention time and sensitivity on [EDTA] are shown in Figure 2-27 and Figure 2-28.

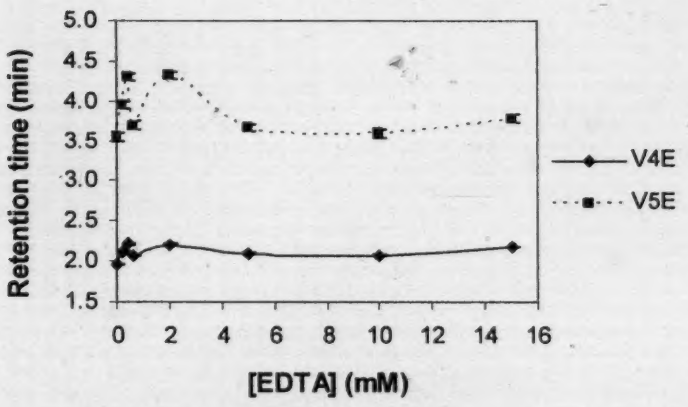

Figure 2-27. Effect of [EDTA] on retention time. Eluent: x mM EDTA, 3% ACN and 80 mM NH_4HCO_3 at pH 6.0

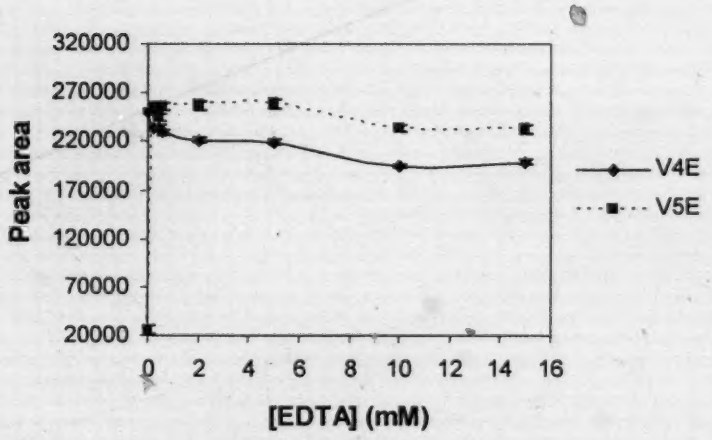

Figure 2-28. Effect of [EDTA] on peak area. Eluent: x mM EDTA, 3% ACN and 80 mM NH_4HCO_3 at pH 6.

As observed in Figure 2-25, without any EDTA in the eluent, the $[VO_2Y]^{3-}$ was very unstable and its peak area was significantly decreased. This is again observed in the decreased peak area at 0 mM EDTA in Figure 2-28. From Figure 2-27, the retention times of V species seem to fluctuate at low concentrations of EDTA. This is probably

due to experimental error associated with weighing small amounts of EDTA. Below 2 mM EDTA, the retention of $[VO_2Y]^{3-}$ seem to be affected slightly more than that of $[VOY]^{2-}$. This is probably due to the larger charge that $[VO_2Y]^{3-}$ carries. To maximize sensitivity, optimize resolution and minimize the amount of chemical introduced into the ICPMS, 2 mM of EDTA was chosen.

2.6.5 Separation of V complexes

To increase resolution, reduce analysis time and maximize sensitivity, the optimum mobile phase was chosen to be 3% ACN, 2 mM EDTA and 80 mM NH_4HCO_3 at pH 6.0 with a flow rate of 1.0 mL/min.

Under these conditions, the three V species (5 µg/L each), including the EDTA complexed V(III), V(IV) and V(V) were separated with the retention times of 0.7 min, 2.4 min and 5.0 min, respectively (Figure 2-29). The dead volume was 0.6 min.

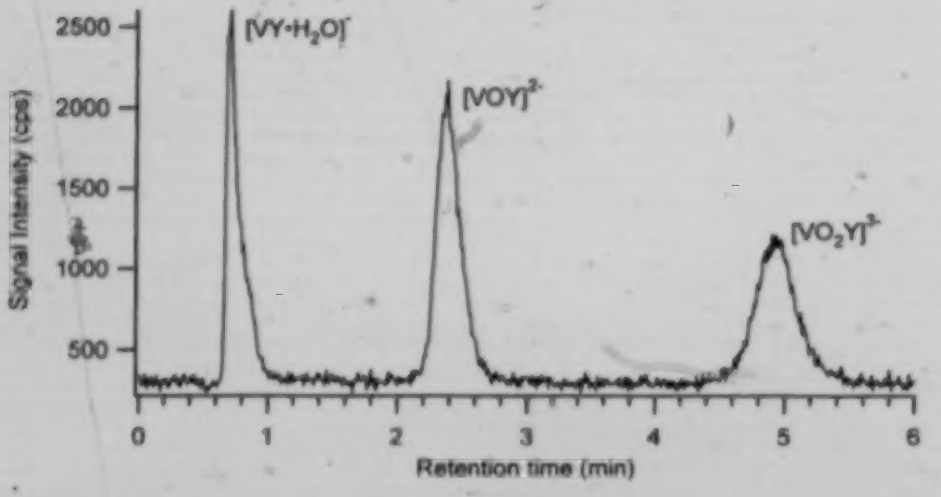

Figure 2-29. Speciation of EDTA complexes of V(III), V(IV) and V(V). Eluent, 3% ACN, 2 mM EDTA and 80 mM NH_4HCO_3 at pH 6.0

2.6.6 Calibration and detection limits

Calibration curves were constructed with five data points for each of the V species over the concentration range 1 μg/L to 100 μg/L. Correlation coefficients were all above 0.999 for all three V species and residuals were random for each plot. Calibration cuves are shown in Figure 2-30.

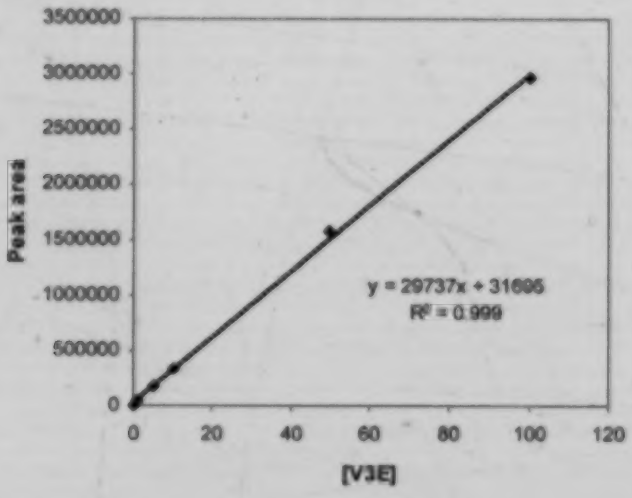

Figure 2-30. Calibration curve for V(III). Eluent, 4% ACN, 2 mM EDTA and 80 mM NH_4HCO_3 at pH 6.0. Data were average of triplicate analyses. Error bars representing ± one standard deviation were smaller than the size of the symbols

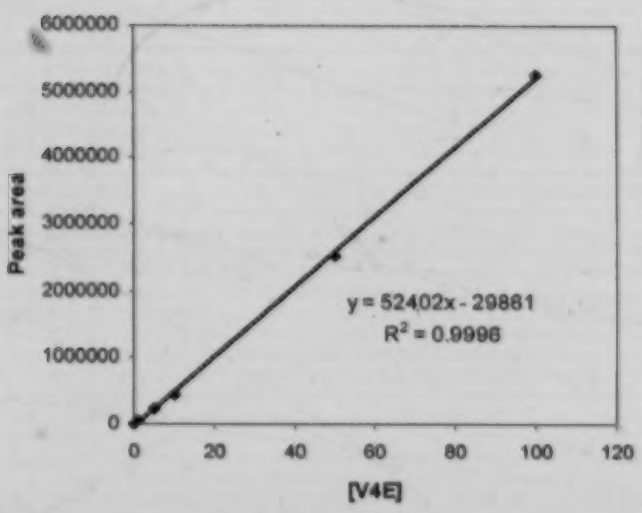

Figure 2-31. Calibration curve for V(IV). Eluent, 4% ACN, 2 mM EDTA and 80 mM NH_4HCO_3 at pH 6.0. Data were average of triplicate analyses. Error bars representing $\pm$ one standard deviation were smaller than the size of the symbols

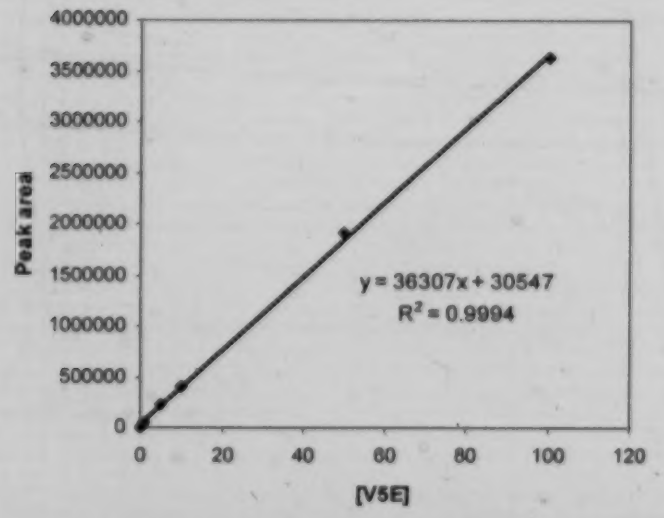

Figure 2-32. Calibration curve for V(V). Eluent, 4% ACN, 2 mM EDTA and 80 mM NH_4HCO_3 at pH 6.0. Data were average of triplicate analyses. Error bars representing $\pm$ one standard deviation were smaller than the size of the symbols

The detection limits were 0.6, 0.7 and 1.0 μg/L for $[VY{\cdot}H_2O]^-$, $[VOY]^{2-}$ and $[VO_2Y]^{3-}$, respectively. The detection limits were based on three times the average background noise for the blank.

The reproducibilities of the method were tested by triplicate analysis of a vanadium mix standard containing 50 μg/L of each of the species. The relative standard deviations were 8.1%, 1.5% and 0.9% for $[VY{\cdot}H_2O]^-$, $[VOY]^{2-}$ and $[VO_2Y]^{3-}$, respectively. The relatively large standard deviation for $[VY{\cdot}H_2O]^-$ is due to its instability in solution. After about 2 min of making up a 250 μg/L sample from the 12 mg/L standard, 8.6% of the peak area for $[VY{\cdot}H_2O]^-$ converted to that of $[VOY]^{2-}$ and $[VO_2Y]^{3-}$.

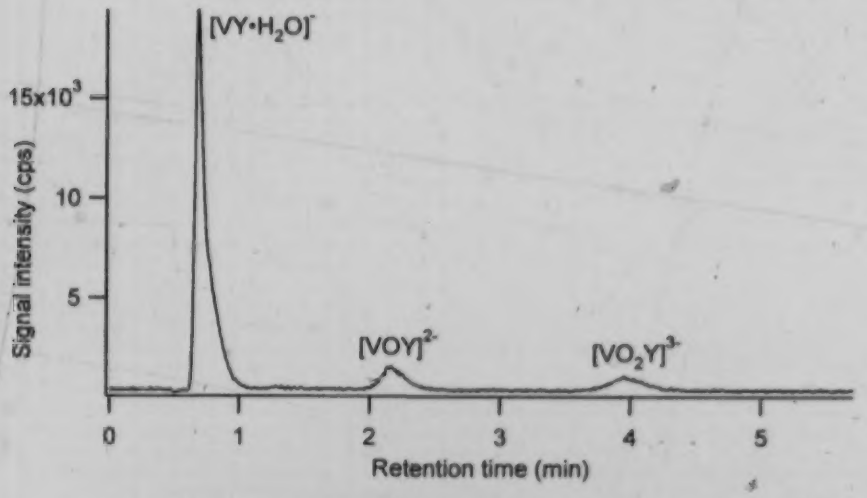

Figure 2-33. $[VY{\cdot}H_2O]^-$ (250 μg/L) after 2 min of dilution. Eluent, 4% ACN, 2 mM EDTA and 80 mM NH_4HCO_3 at pH 6.0

The synthesized $[VY{\cdot}H_2O]^-$ complex was the least stable species out of the three vanadium species. Six months after its synthesis, the complex was dissolved in DIW and diluted to make a 20 μg/L solution. When analyzed on the HPLC-ICPMS, the V(III)

peak was observed to decrease whereas the V(IV) peak increased rapidly with time (Figure 2-34). This conversion rate was slower when the $[VY{\cdot}H_2O]^-$ complex was less aged. The V(V) peak remained unchanged, suggesting that V(III) oxidized to V(IV) in solution.

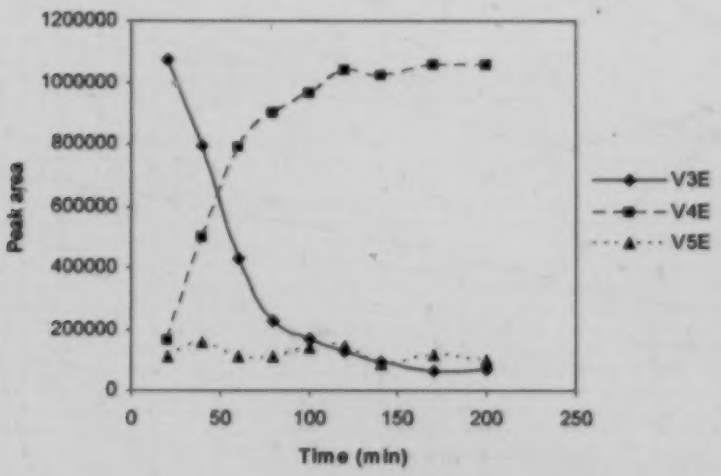

Figure 2-34. Dependence of the stability of $[VY{\cdot}H_2O]^-$ on time

With this sample of $[VY{\cdot}H_2O]^-$, the conversion of V(III) to V(IV) seemed to be complete in 3 h, and the ratio of the peak areas of V(III) to V(IV) was constant even after 9 d of making up the 20 µg/L standard.

2.6.7 Interferences

There were no significant interferences with the V signals. The only isobaric interference was ClO^+ interfering with ^{51}V, but it is separated from the three V species. Chloride elutes at 1.1 min immediately after $[VY{\cdot}H_2O]^-$, and before $[VOY]^{2-}$ (Figure 2-35). The fact that Cl^- is retained longer than $[VY{\cdot}H_2O]^-$ is probably because the small

electronegative atom interacts significantly with the cationic quaternary ammonium stationary phase.

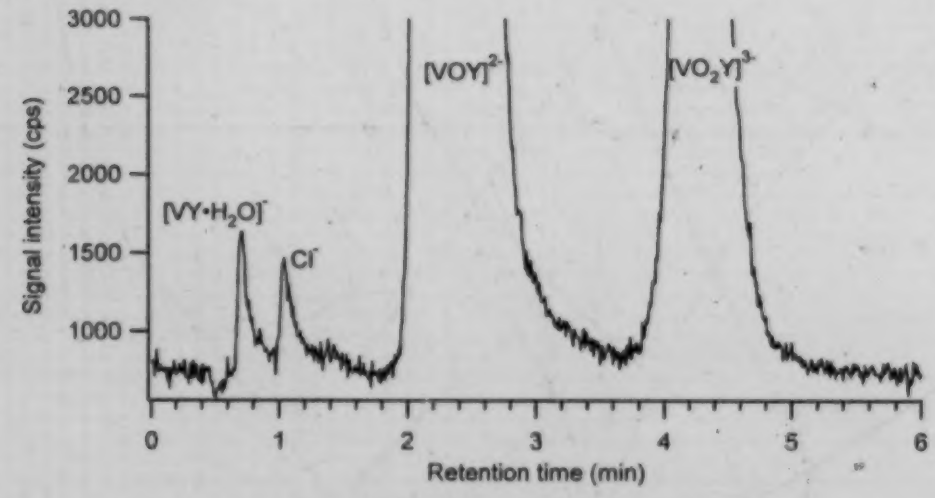

Figure 2-35. Separation of V(III) from potential Cl^- interference

In conclusion, a HPLC-ICPMS method has been developed using the SAX column PRPX100 to efficiently separate V(III), V(IV) and V(V) as EDTA complexes in about 5 min. The optimum mobile phase consists of 3% ACN, 2 mM EDTA and 80 mM NH_4HCO_3 at pH 6.0. The method has high sensitivity and no significant interferences.

2.7 References

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8. Jen, J. and Yang, S. Simultaneous speciation determination of vanadium(IV) and vanadium(V) as EDTA complexes by liquid chromatography with UV detection. *Anal. Chim. Acta* **1994,** *289,* 97-104.

Chapter 3. Application of Method

Two different types of samples were investigated in this research to test the suitability of the method for analyzing real samples. The first type was coke water samples. Speciation analyses were done on samples collected in the field as well as samples generated in the laboratory to assess the behavior of vanadium in the environment. The second type was *S. putrefaciens* CN32 bacteria incubated with V(V) for different durations of time. These samples were analyzed to see if the bacteria could reduce V(V) to the less toxic, or lower oxidation states of V. This is the first time that V speciation studies have been done for both types of samples.

3.1 Coke Pore Water Samples

3.1.1 Background

In Alberta, the oil sand deposits offer a major resource for economic development. However, the waste product from the oil refinery process, coke, is produced at an alarming rate that is causing concern. Since it can still potentially be used as an energy source in the future, coke is stored away in lysimeters on reclamation lands. Lysimeters are cylindrical coke tanks capped with an upper soil layer with colonizing biota. At these sites, coke is placed as a layer between the oil sands process-affected materials (OSPM) and the reclamation soil layer in which plants will be grown. Test sites on selected beaches at Syncrude's Mildred Lake Settling Basin (MLSB) have been constructed in partnership with the University of Saskatchewan.

Little is known of how much vanadium is stored in the cokes or how mobile it is with infiltration waters. Even less is known about the speciation of V in these types of waters, since the metal has not been a concern until recently. In the following studies, these issues were addressed and by the results, the issues about placing coke into a reclamation environment could be elucidated.

3.1.2 Overview of experiments

To evaluate the role of natural leaching through infiltration of precipitation through the deposited petroleum coke, water samples were collected from Syncrude's MLSB coke beach. Lysimeter coke pore waters were collected from coke tanks implanted on the beach around 2002 that were capped with soil the following winter. These sites are exposed to precipitation and collected water is pumped from them periodically for environmental monitoring.

Prior to depositing the coke on the reclamation lands, wells were installed near coke cells to draw process water out of coke. Some of the water samples from these wells were also analyzed to see how much and what forms of vanadium have accumulated over the years.

Besides analyzing coke pore waters from the coke deposits at Syncrude, several experiments were done in the laboratory to compare how fast V leaches from various sources. These include Syncrude Fresh Coke (SFC), Syncrude Aged Coke (SAC) and Suncor Delayed Coke (SDC). The speciation of V in the leachates from these three types of coke was compared.

In reconstructed wetlands, there is the potential for coke to be exposed to various pH and dissolved oxygen (DO) concentrations due to layering with other waste products

such as tailings. To explore the effects of pH and DO, different environmental conditions were simulated in the leaching water that was used to extract V from the cokes. These experiments were designed to evaluate the kinetics of V release to be expected in the natural environment. They will allow us to understand how the species of V change under various conditions and how much of V is made available to biota.

3.1.3 Lysimeter samples

Vanadium is detected at a wide range of concentrations (< 0.1 mg/L to 12 mg/L) on Syncrude's reclamation sites. To get an idea of speciation of V in this range, three samples were collected: two from lysimeter sites with elevated amount of V and the other from a pond with almost non-detectable concentrations of V. The samples of infiltration water from the oil refinery site were collected at sub-zero temperatures in February of 2006. The lysimeters are large cylindrical tanks (2.5 m in depth, 2.44 m in diameter) with coke stored on the bottom and a layer of soil capped on the top for reclamation purposes. The lysimeter water samples were collected from the lower 30 cm of the tanks beneath a shallow soil cap (SP040460) and beneath a deep cap (SP040461). The former site is capped with 35 cm of soil whereas the latter with 100 cm of soil (Figure 3-1).

A sample from the non-detectable site was from a man-made wetland, or peat pond (PP1). This pond is 2 m deep and the water was collected below 25 cm of ice (1). All the samples were filtered through 0.45 μm filters before they were shipped to the laboratory.

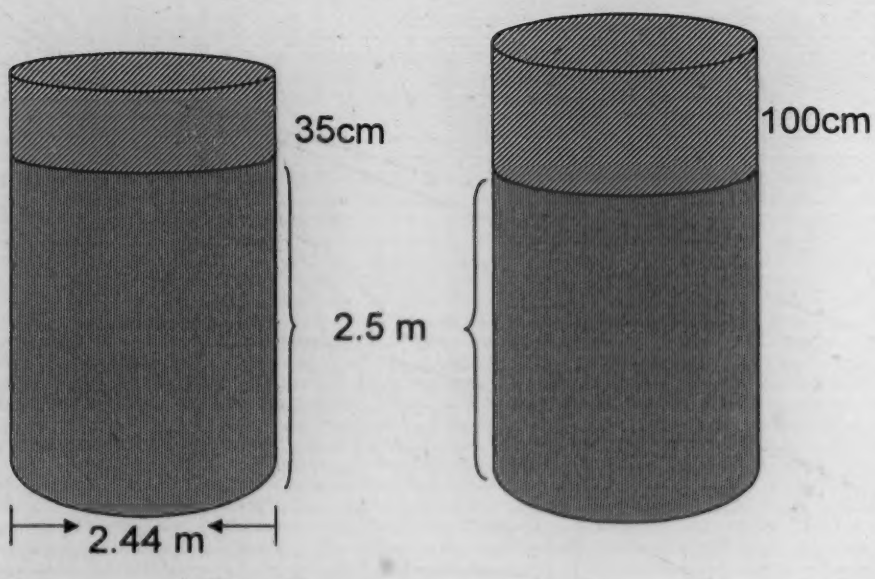

Figure 3-1. Layout of two lysimeters. The cylinders contain an upper layer of soil and a bottom layer of coke

When the samples arrived in the laboratory 10 d after their collection, excess EDTA acid (twice the molar ratio to V) was added to the samples and they were filtered through a 0.45 μm filter and diluted 10 times with DIW. The samples were analyzed with 4% ACN, 15 mM EDTA and 80 mM NH_4HCO_3 at pH 6.0 on the SAX column. Figure 3-2 shows the chromatograms for the three samples and Table 3-1 shows the concentrations of V species found in each sample.

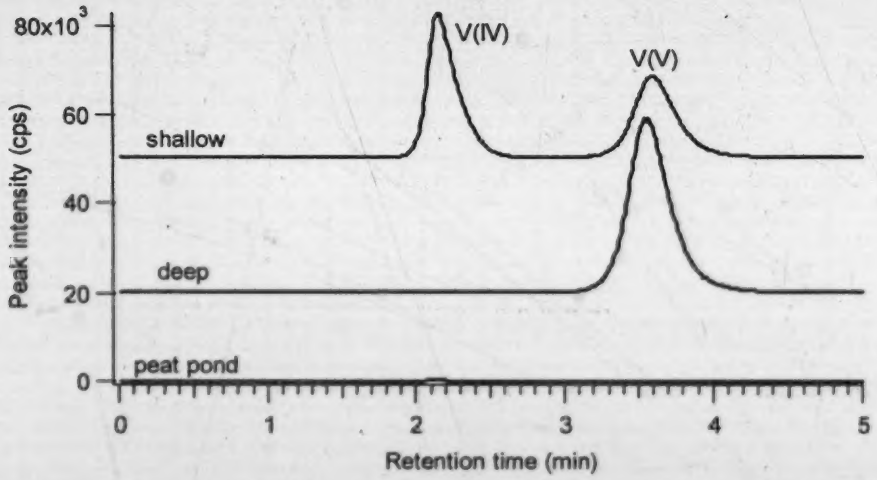

Figure 3-2. Chromatograms of water samples from Syncrude's reclamation sites. The samples were analyzed with 4% ACN, 15 mM EDTA and 80 mM NH_4HCO_3 at pH 6.0 on the SAX column

Table 3-1. V speciation in lysimeter and pond water samples

Sample	Descript.	EC (dS/m)	pH	[V(IV)] (μg/L)	[V(V)] (μg/L)	[V]total (μg/L)
SP040460	Shallow	1.39	6.46	$1.1x10^3$	$9.3x10^2$	$2.0x10^3$
SP040461	Deep	1.35	6.84	$4.8 x10^1$	$5.1x10^3$	$5.2x10^3$
PP1	Peat pond	1.35	7.04	$1.1x10^2$	$3.0x10^1$	$1.4x10^2$

Three days after analyzing the sample, any V(IV) originally present was found to have fully oxidized to V(V) in samples stored in the 4°C fridge.

From the above data, it is seen that there is more V(V) at the deep site. This could be due to a higher pH that results in a more oxidized form of the V species. This would be consistent with the effect of pH observed in the previous chapter (Section 2.5.1). Another reason could be that the dissolved oxygen (DO) concentration was higher at the deep site. However, DO concentrations were not measured and this can only be hypothesized.

Five months later, samples from the same lysimeter sites were collected in July of 2006 and the data in Table 3-2 were obtained:

Table 3-2. Speciation of V in coke pore waters collected on two different days

Collected on	Cap depth	pH	[V(IV)] (μg/L)	[V(V)] (μg/L)
Feb 13, 06	Shallow	6.46	1.1×10^3	9.3×10^2
	Deep	6.84	48	5.2×10^3
July 19, 06	Shallow	7.43	BDL	1.8×10^3
	Deep	7.09	BDL	4.1×10^3

The pH seems to have been elevated in these samples later in the year, resulting in the observation of only the V(V) species. This is probably due to the fact that a higher temperature increased the kinetics of the oxidation. It is again observed that the deep-capped site leaches out more V than the shallow site. This could be due to the fact that more water is retained in the deep cap and less water is percolating through the coke layer below, causing V to be more concentrated at this site. To explore this possibility, different amounts of DIW (10, 20, 35, 50 and 100 mL) were added to 25 g samples of SAC. The coke slurries were allowed to equilibrate for 90 min and filtered through a 0.45 μm filter. Excess EDTA acid was added to 5 mL of each sample which was allowed to equilibrate for 20 min afterwards.

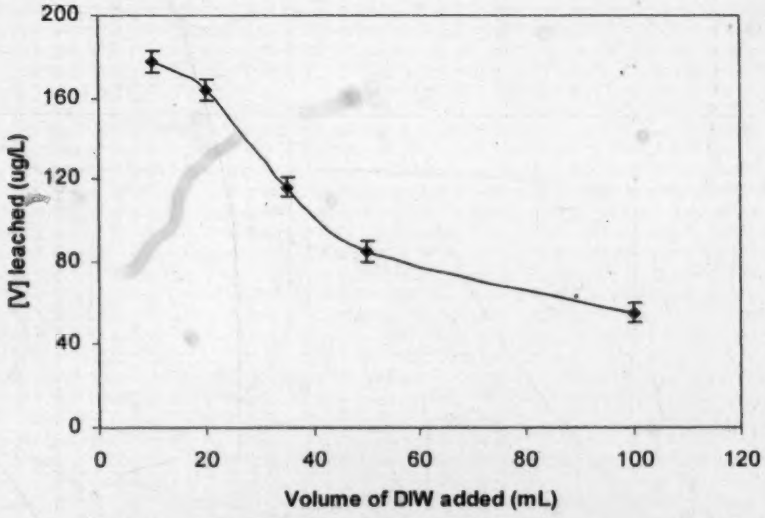

Figure 3-3. Total concentration of V leached from 25 g of SAC vs volume of DIW added. Error bars are based on the standard deviation of a triplicate injection

As shown in Figure 3-3, when less DIW is combined with coke, a higher concentration of leached V is observed since there is little dilution. However, when we look at the mass of V leached out versus the volume of DIW added, the opposite trend is observed (Figure 3-4). The mass of V is calculated by multiplying the [V] and the volume of DIW added to the sample. With the volume of DIW taken into account, a higher amount of V is actually observed to leach into solution with a higher volume of DIW. This makes sense since we expect higher volumes of DIW to leach out more soluble constituents.

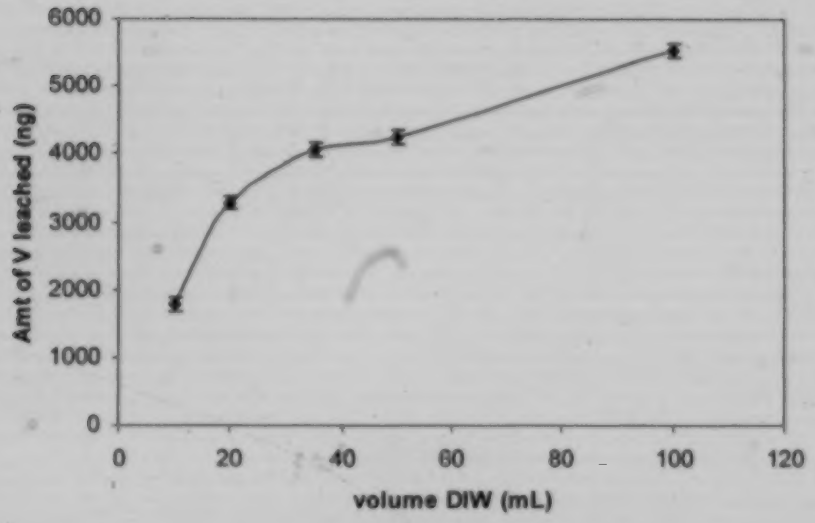

Figure 3-4. Mass of V leached from 25 g SAC vs. volume of DIW added. Error bars are based on the standard deviation of a triplicate injection

3.1.4 Coke cell samples

Prior to storing coke on reclamation lands, wells in coke cells were constructed to dry slurried coke. Several dewatering wells were placed near the center of the coke cells to pump process water out of slurried coke. In an effort to monitor the effect of the process water on the environment, waters from these wells are continuously monitored. In this experiment, water samples from two wells located near Coke Cell #5 collected at two different times in the summer were analyzed. Coke Cell #5 consists of a field of more than 9 hectares of slurried coke on the reclamation land at Fort McMurray. Table 3-3 shows the speciation of V found in each. The motivation for analyzing these samples was that there is a lake located nearby Coke Cell #5 and there is concern regarding potential environmental contamination.

A groundwater well (CC5 SP05CC06) is a standpipe piezometer installed on the east side of Coke Cell #5 and the coke from this well has been in contact with water for about 10 years. CC5 well #2 and CC5 well #5 are large dewatering wells installed near the center of the coke cell. Water is continuously being pumped out of these wells. Well #2 has a 10 m screen with the bottom of the well located at 28 m below the coke surface. Well #5 also has a 10 m screen with the bottom of the well located at 46 m below the coke surface. The pumped water samples were collected drop by drop from the wells (2), therefore, there was lots of time for oxidation to occur. When the samples were received, the groundwater well had fine powder of coke suspended in it whereas the two samples from the dewatering wells were clear. Vanadium concentrations in these samples were analyzed and compared.

Table 3-3. Speciation of V in coke cells

Collected on	Source	pH	[V(IV)] (µg/L)	[V(V)] (µg/L)
July 19, 06	CC5 well#2	8.3	BDL	7.7
	CC5 well#5	8.4	1.05	6.6
	CC5 SP05CC06	8.3	BDL	750
Aug 1, 06	CC5 well#2	8.1	BDL	8.3
	CC5 well#5	8.1	BDL	6.1
	CC5 SP05CC06	8.2	BDL	770

The concentration of V observed in Wells #2 and #5 (Table 3-3) were much lower than the concentrations found in the lysimeters (Table 3-2). This is likely due to the fact that the water is continuously pumped out from these wells. New process water that has just been added to the coke and did not have much time to leach out V before it was pumped out. Comparing values from Table 3-3, CC5 SP05CC06 contains around 100-fold more V than the well water samples. This could be due to the long contact time between coke and these water samples. In addition, there is coke suspended in this

sample, causing more V to leach out due to the greater contact of water with the suspended coke. Most of the V present in all the wells is in the form of V(V) due to oxidation and the high pH of these waters.

3.1.5 Leaching studies

Over time on the reclamation land, coke will be repeatedly exposed to rain, snow and other elements in nature. To study the rate and amount of V leached from coke by infiltration waters, repeated leaching studies have been done by extracting one sample of 25 g of SDC with 50 mL portions of water or a salt solution. For each extraction, the slurried coke was allowed to equilibrate overnight before being filtered by a 0.45 μm filter the next morning.

To see how many extractions are required to extract most of the V out of coke and whether there is a difference between leaching with water or with a salt solution resembling rain water, two leaching profiles were generated (Figure 3-5). The salt solution contained components resembling those of rainwater found in the samples from the reclamation sites (Table 3-4). The salt solution was made up by dissolving 0.73 mg/L $(NH_4)_3PO_4$, 320 mg/L $NaHCO_3$, 594 mg/L $MgSO_4$ and 80 mg/L $CaCl_2$ in DIW.

Table 3-4. Salts found in lysimeter samples sampled on two days

Ion (mg/L)	Peat pond	Shallow capped	Deep capped
Na^+	140/130[a]	280/260	280/260
K^+	0.1	5.3	0.1
Mg^{2+}	33/30	10/12	5.7/13
Ca^{2+}	80/73	15/18	7.3/14
Cl^-	32/30	6.8/7.2	7.6/8.0
SO_4^{2-}	330/290	560/590	480/520
HCO_3^-	330/320	67/65	150/130
PO_4^{3-}	BDL	N/A	N/A
NH_4^+	0.31	0.34/0.73	N/A

[a]The two values correspond to salt concentrations found in samples collected on February 13 and 14, 2006.

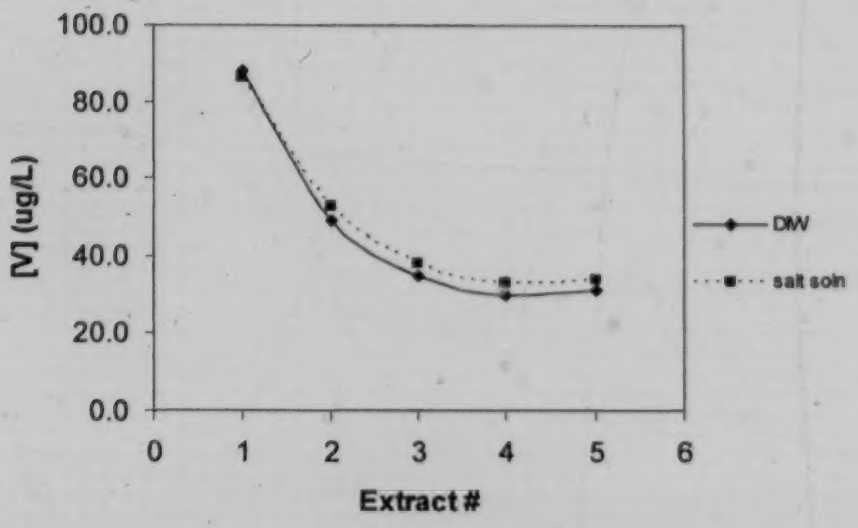

Figure 3-5. Leaching profiles of V leached from SDC with DIW and salt solution

From this experiment, the only species of V observed in the chromatogram is V(V). From the above figure, it seems that the concentration of extracted V plateaus after four extractions. A paired-sample t-Test found that the salt solution possibly leaches out slightly more V than DIW, but the difference is inconclusive.

Also, upon examining the chromatograms from the second and third extracts from the salt solution closely, a small but visible peak at 1.5 min is observed (Figure 3-6). This peak could have resulted from V bonding with salt(s) in the solution.

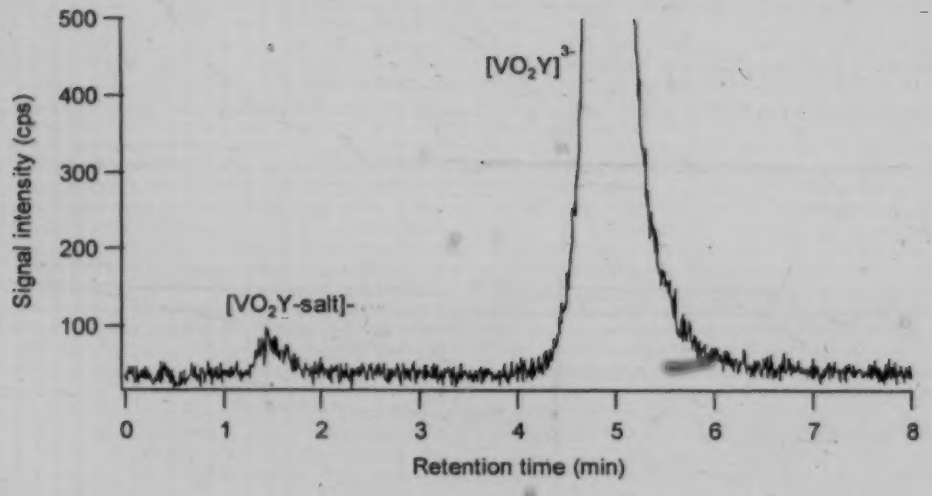

Figure 3-6. Chromatogram of the third extract from salt solution extraction

3.1.6 Interaction of V and salts

At first glance, this peak at 1.5 min might be suspected for being V(IV). This is not the case because according to the chromatogram of the standard (Figure 3-7), $[VOY]^{2-}$ elutes at 2.3 min. Since there is only V(V) present in the leachates, the peak at 1.5 min is probably due to $[VO_2Y]^{3-}$ binding to a divalent cationic salt, resulting in a singly charged negative ion. Out of curiosity for the identity of the cation, the following experiments were done.

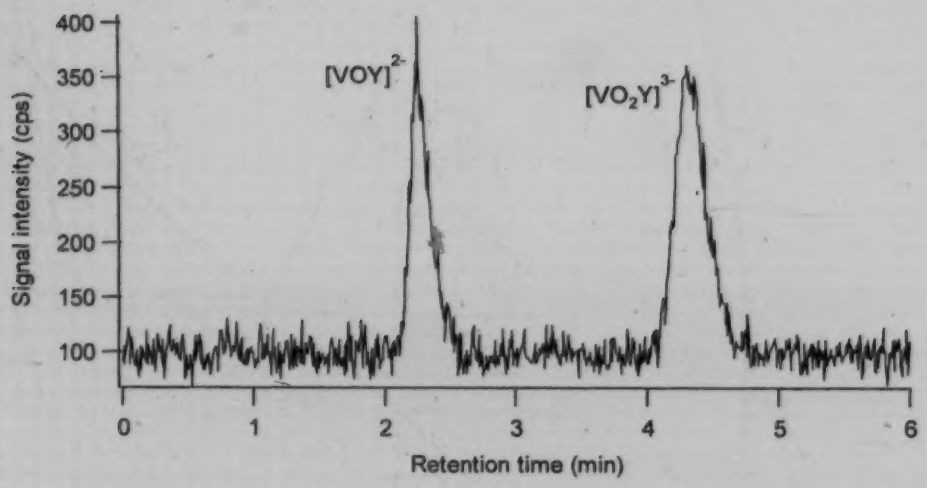

Figure 3-7. A mix of the $[VOY]^{2-}$ and $[VO_2Y]^{3-}$ standards at 5 µg/L each

From the list of all the ions found in the salt solution (Table 3-4), the only divalent cations are Mg^{2+} and Ca^{2+}. To see if they could possibly bind to $[VO_2Y]^{3-}$, excess concentrations (2.38 g/L Mg^{2+} and 0.34 g/L Ca^{2+}) of each were made up. In addition, 1.34 g/L Na^{+} was made up since it is a major ion in the salt solution. The pH values for all the salt solutions were adjusted to 6.0. Each salt solution was added to the V(V) standard in a 1:1 ratio and allowed to equilibrate with a mix of the $[VOY]^{2-}$ and $[VO_2Y]^{3-}$ standards (50 µg/L each) overnight. In the sample of $[VO_2Y]^{3-}$ incubated with Na^{+}, only the $[VO_2Y]^{3-}$ peak was observed. This implies that there is no interaction between the two ions.

In the sample of $[VO_2Y]^{3-}$ incubated with Mg^{2+}, there seem to be some interactions since several small peaks (Figure 3-8) appeared in the baseline.

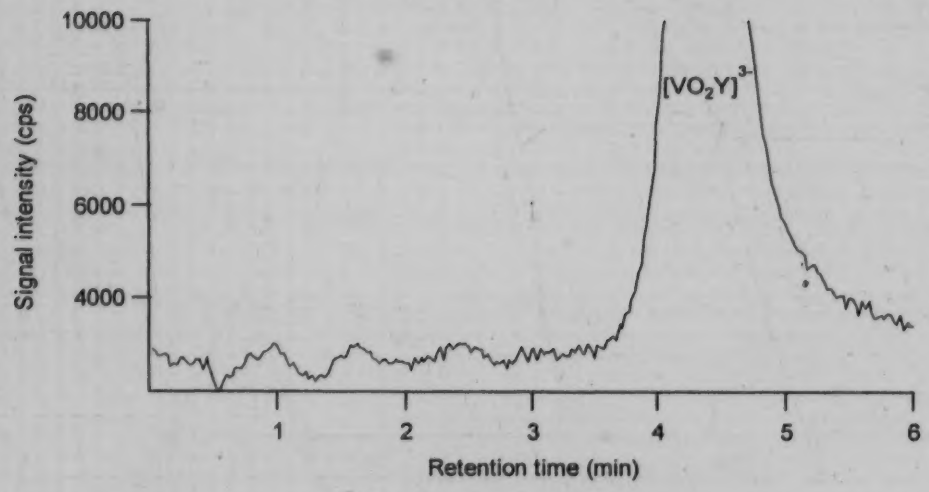

Figure 3-8. $[VO_2Y]^{3-}$ (25 μg/L) in 2.38 g/L Mg^{2+} solution

After equilibrating $[VO_2Y]^{3-}$ with Ca^{2+} overnight, a new peak at 1.5 min appeared in the $[VO_2Y]^{3-}$ standard as shown in Figure 3-9.

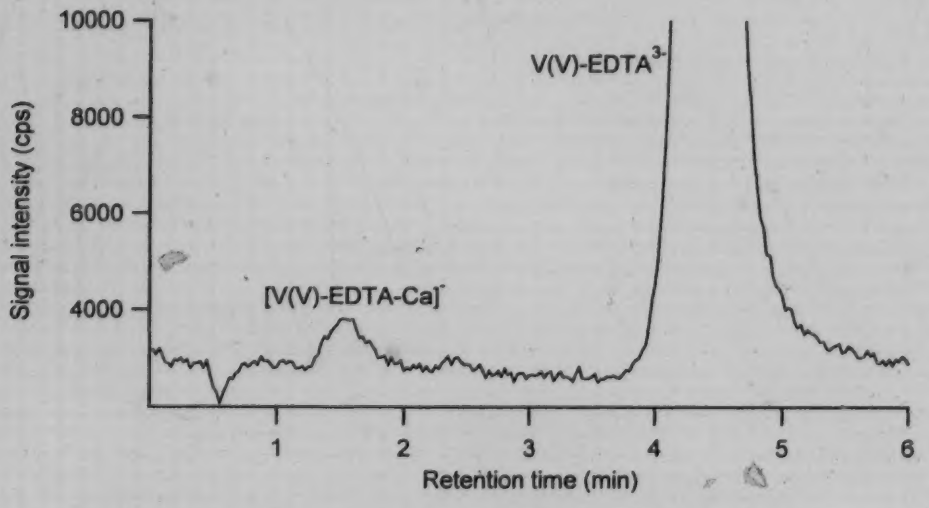

Figure 3-9. $[VO_2Y]^{3-}$ (25 μg/L) in 0.34 g/L Ca^{2+} solution

Thus, the peak at 1.5 min can most likely be attributed to $[VO_2Y]^{3-}$ binding with Ca^{2+}. From literature, it is known that V(V) resembles phosphate structurally (VO_4^{3-} vs

PO_4^{3-}) and it interacts with cationic amino acids via H-bonding and Zn^{2+} via polar interactions (3). It seems like $[VO_2Y]^{3-}$ also interacts with Mg^{2+} and Ca^{2+} through polar interactions. In experiments done with inorganic V(V) incubated with Mg^{2+} and Ca^{2+}, similar interactions were observed.

3.1.7 Comparing leaching profiles

To get back to the question of how many extractions it takes to leach V out of coke, three types of coke were analyzed – SFC, SAC and SDC. A Na_4EDTA solution (2.5 mM) was deoxygenated by purging with N_2. One hundred milliliters of this solution was added to 25 g of each type of coke. The mixtures were sealed and allowed to equilibrate overnight. They were filtered through a 0.45 μm filter the next morning. They were subsequently centrifuged, diluted and analyzed. The procedure was repeated six times at which point a negligible amount of V was leaching from the coke.

After analysis, the amount of V leached per gram of coke was plotted against extraction number (Figure 3-10), which is directly related to time.

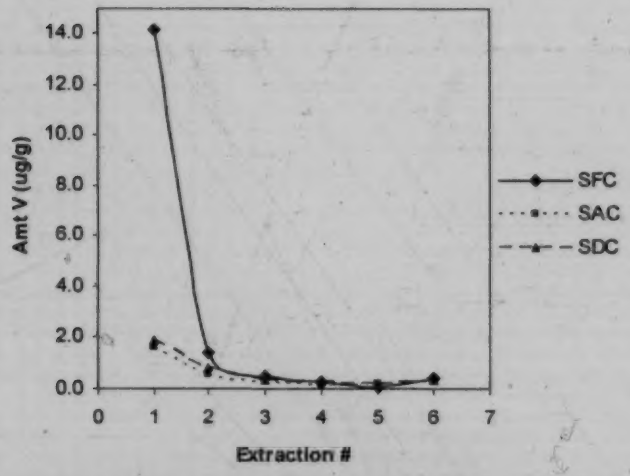

Figure 3-10. Leaching profiles of the three types of coke. Coke (25 g) was extracted with 100 mL of Na_4EDTA solution (2.5 mM)

Figure 3-10 shows that SFC leaches out the greatest amount of V, especially in the first extraction. From a paired-sample t-Test done to compare SDC and SFC, a suggestive but inconclusive difference was found in the amount of V that is leaching between these two types of coke.

In the six extractions, SFC leached 16.8 μg of V per gram of coke, while SDC leached 3.8 μg/g and SAC leached 3.1 μg/g. Eighty percent or more of V was extracted in the first three extractions for all three types of coke. In fact, after the third extraction, the leaching profiles for all three types of coke appeared approximately the same.

Figure 3-10 shows the leaching profile for total V, but different species of V were also observed when the leachates were analyzed. In Figure 3-11, it seems that SDC leaches out the most V(IV). SAC has been in contact with water and any V(IV) present in it is fully oxidized, therefore no V(IV) is observed to leach from SAC.

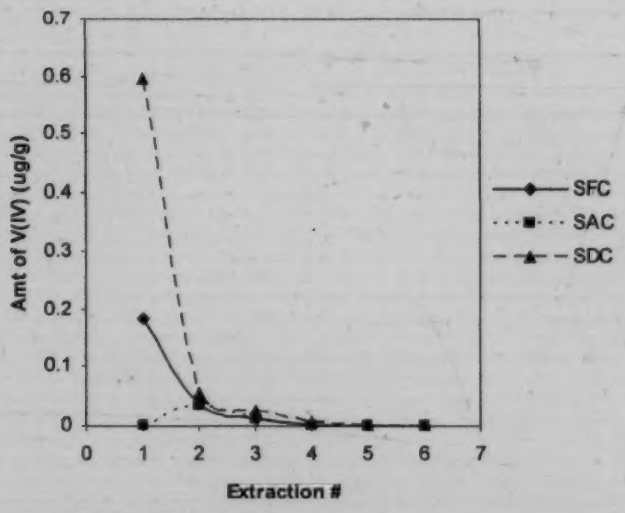

Figure 3-11. Leaching profile for V(IV) among three cokes

The leaching profile (Figure 3-12) for V(V) is similar to that for total V, with SFC leaching out the most V(V), most significantly in the first extraction.

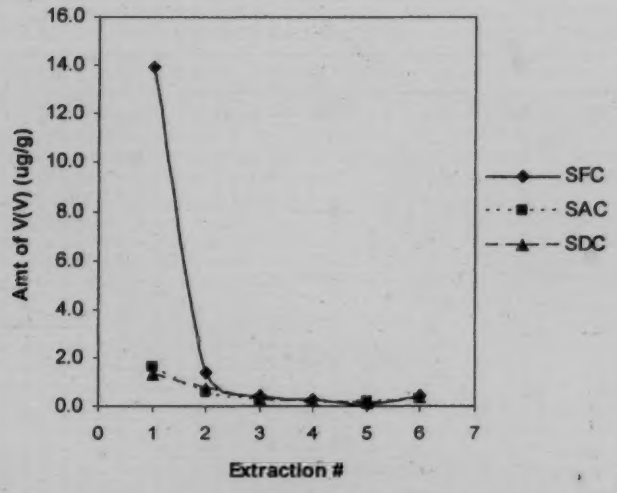

Figure 3-12. Leaching profile for V(V) among three cokes

From the speciation data above, it seems that SAC is the most oxidized form of coke since it has been slurried with water for transportation. On the other hand, SDC is the least oxidized. This is consistent with the moisture content and physical characteristics data for SDC presented in Section 1.7.1.

3.1.8 Effect of pH on V speciation in coke leached water

The importance of pH to V speciation was shown in Section 2.6.1. In reconstructed wetlands, there is the potential for coke to be exposed to various pH levels due to layering with other waste products such as tailings. On the reclamation lands, coke can also be exposed to precipitation which could have different pH levels. Squires (6) from the University of Saskatchewan has explored the effect of pH on the total concentration of several metals leached from coke, but no speciation data is yet available for any of the metals studied.

To observe the effect of pH on V speciation, SFC, SAC and SDC were exposed to three levels of pH: 5, 7.5 and 10. First, three containers each containing 400 mL of 2.0 mM Na_4EDTA solutions were prepared. The pH of each solution was adjusted to 5, 7.5 and 10 with HNO_3 and NH_4OH. Each leaching solution was sparged with N_2 for 2 h to prevent aerial oxidation in the samples. Each solution (100 mL) was mixed with 25 g of each coke in Nalgene bottles. The bottles were sealed and allowed to equilibrate overnight. The next morning, overlying water was carefully decanted off the coke layer and filtered. The remaining pore water was extracted from the coke by vacuum filtration with a 0.45 μm membrane filter. These two types of waters were analyzed on the HPLC-ICPMS separately. The same procedure was repeated on two other days to assess reproducibility.

Table 3-5 summarizes the concentrations of V that were obtained under each pH condition for each type of coke.

Table 3-5. Total vanadium concentration from pH experiment (μg/L)

Coke type	pH (±0.1)	Overlying water	Pore water
SFC	5.0	1600 ± 300	5400 ± 700
SFC	7.5	1800 ± 500	4900 ± 700
SFC	10.0	1800 ± 400	5600 ± 600
SAC	5.0	200 ± 200	900 ± 300
SAC	7.5	220 ± 90	700 ± 200
SAC	10.0	220 ± 110	1000 ± 800
SDC	5.0	80 ± 50	100 ± 100
SDC	7.5	70 ± 50	100 ± 60
SDC	10.0	80 ± 60	100 ± 100

It is known that under lower pH conditions, most metals tend to be released into water from sediment more easily (4). It has also been shown in previously conducted short-term work that coke may produce acutely toxic seepage water under more acidic conditions (5). From the above data, it seems that not only is V leached into the water at low pH (pH 5.0) as Wren and Stephenson (4) proposed, a significant amount is also leached into the water at high pH (pH 10.0) as well.

From Table 3-5, it is apparent that V is more concentrated in the pore water fractions. But this does not tell us whether there is more V in the pore water or whether V is simply diluted by the larger volume of water in the overlying water. It has been found previously that 10 mL of DIW are required to saturate 25 g of coke. Thus it can be approximated that there are 90 mL of overlying water and 10 mL of pore water in each sample. With the volume of water taken into account, the mass of V can be deduced. Table 3-6 shows the mass of V in each type of water from each coke.

Table 3-6. Mass of total vanadium from pH experiment

Coke type	pH (±0.1)	Overlying water (μg)	Pore water (μg)
SFC	5.0	150 ± 30	50 ± 10
SFC	7.5	160 ± 50	49 ± 7
SFC	10.0	160 ± 30	60 ± 10
SAC	5.0	20 ± 20	9 ± 3
SAC	7.5	20 ± 8	7 ± 2
SAC	10.0	20 ± 10	10 ± 8
SDC	5.0	7 ± 5	1 ± 1
SDC	7.5	6 ± 4	1 ± 1
SDC	10.0	7 ± 5	1 ± 1

Similar to what we observed in Section 3.1.2, even though V is more concentrated in the pore water, a higher amount of V is actually leached into the overlying water which is associated with more DIW.

Figure 3-13 to Figure 3-15 show the relationship between V speciation and pH. The percent of V plotted on the y-axis is calculated from peak areas of V(IV) and V(V).

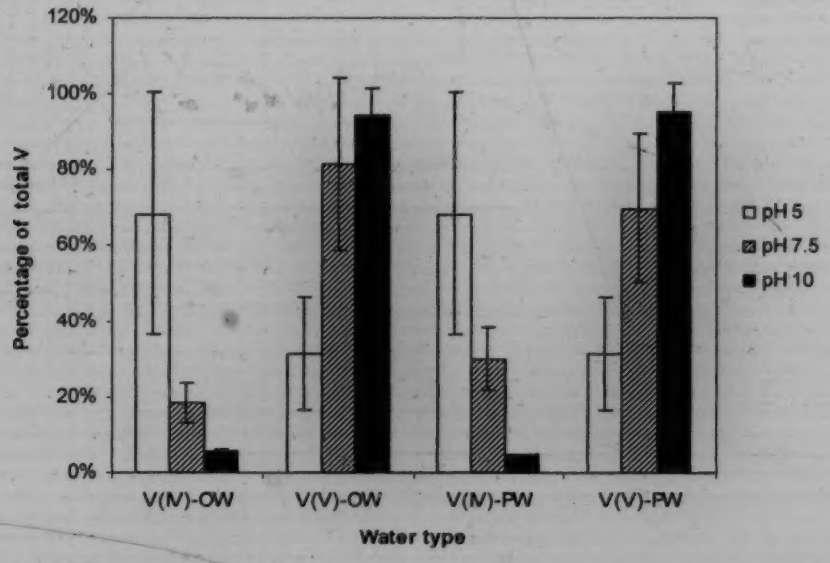

Figure 3-13. Effect of pH on V speciation in SFC. OW denotes overlying water and PW denotes pore water. The error bars denote standard deviation of three experiments run on separate days

As expected from results obtained from Section 2.6.1, when pH increases, the amount of V(IV) decreases whereas V(V) increases since oxidation occurs at higher pH. In the study done with SAC, the same trend is observed with increasing pH and this is shown in Figure 3-14.

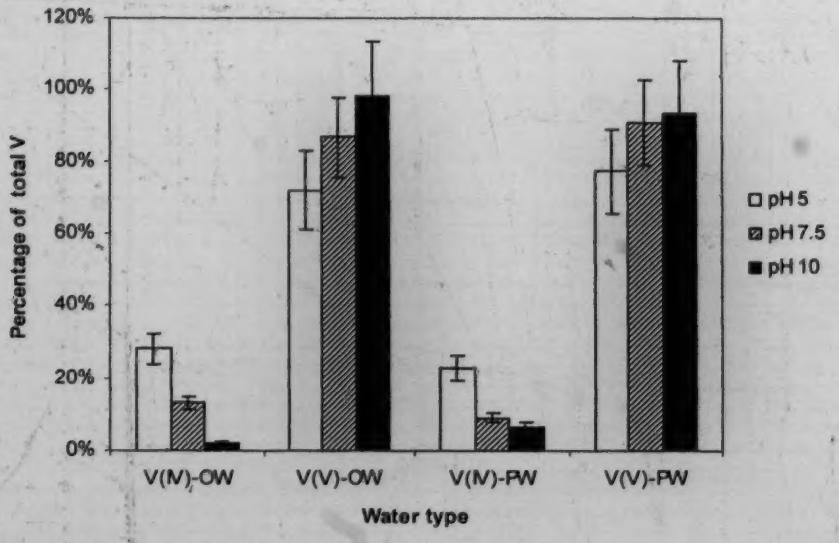

Figure 3-14. Effect of pH on V speciation in SAC. OW denotes overlying water and PW denotes pore water. The error bars denote standard deviation of three experiments

According to Figure 3-14, SAC displays the same trend as SFC. However, we do not see a significance presence of V(IV). This is likely due to the fact that the aged coke has been oxidized.

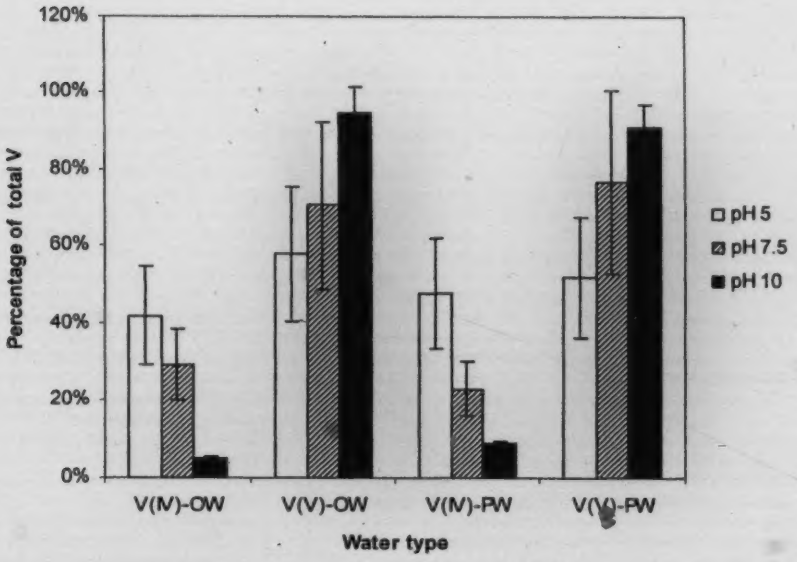

Figure 3-15. Effect of pH on V speciation in SDC. OW denotes overlying water and PW denotes pore water. The error bars denote standard deviation of three experiments

Again, the same trend of the effect of pH is observed in SDC (Figure 3-15). Comparing the percentages of V(IV) in the OW at pH 5 amongst the three cokes, we can see that SAC is the most oxidized form of coke, or it contains the least V(IV). The second most oxidized form of coke is SDC, followed by SFC. These differences in speciation are expected from the characteristics of cokes as described in the section on the background of coke (Section 2.1.2). Syncrude coke in general has higher moisture content than Suncor coke except for SFC, which has the lowest moisture content since it comes out of the coker at 600°C.

3.1.9 Effect of dissolved oxygen on cokes

Another important variable to consider is the DO in the leaching water. Water associated with coke from different sources on the reclamation land will contain different

amounts of DO, which would possibly affect the speciation of V in them. This then would affect the relative toxicities of these waters.

In this part of the study, two 400 mL Na_4EDTA solutions (2.0 mM each) were prepared and their pH adjusted to 6.0 using HNO_3 and NH_4OH. The solutions were each sparged with O_2 or N_2 for 3 h. DO concentrations were measured with a DO probe to be 6.47 ± 0.18 mg/L and 3.09 ± 0.48 mg/L in the O_2-treated water and N_2-treated water, respectively. Each type of water (100 mL) was combined with 25 g each of SFC, SAC and SDC and mixed thoroughly in plastic Nalgene bottles. Each bottle was then sealed and allowed to equilibrate overnight. The overlying water and pore water were filtered and analyzed separately. The total concentrations of V obtained under each condition are given in Table 3-7.

Table 3-7. Total vanadium concentration in DO experiment (μg/L)

Coke type	O_2 level	Overlying water	Pore water
SFC	High O_2	1700 ± 400	26000 ± 1000
SFC	Low O_2	1770 ± 90	30000 ± 20000
SAC	High O_2	340 ± 70	1000 ± 100
SAC	Low O_2	350 ± 30	900 ± 200
SDC	High O_2	16 ± 9	18 ± 6
SDC	Low O_2	20 ± 20	30 ± 20

Comparing the cokes, SFC leaches out more V than the other two kinds whereas SDC leaches out the least amount of V. The low maximum dry density of SDC (Section 2.1.2) does not allow it to mix homogeneously with water. The coke is actually hydrophobic such that when it is combined with water, it floats on top of the solution. This property of SDC does not allow the coke to have sufficient contact with water in order to leach out significant amounts of V.

The trends observed for V treated with water containing different amounts of O_2 are shown in Figure 3-16 to Figure 3-18.

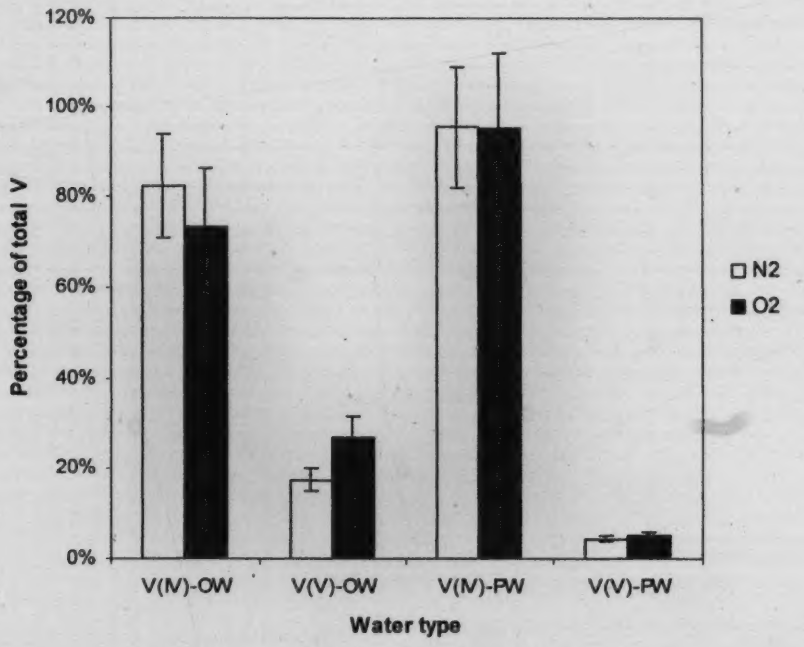

Figure 3-16. Effect of DO concentration on V speciation in SFC. OW denotes overlying water and PW denotes pore water. The error bars denote standard deviation of three experiments

T-tests were done to compare the percentages of each type of V in N_2-treated samples and the O_2-treated samples. No significant difference between the two treatments was found in any of the samples in SFC.

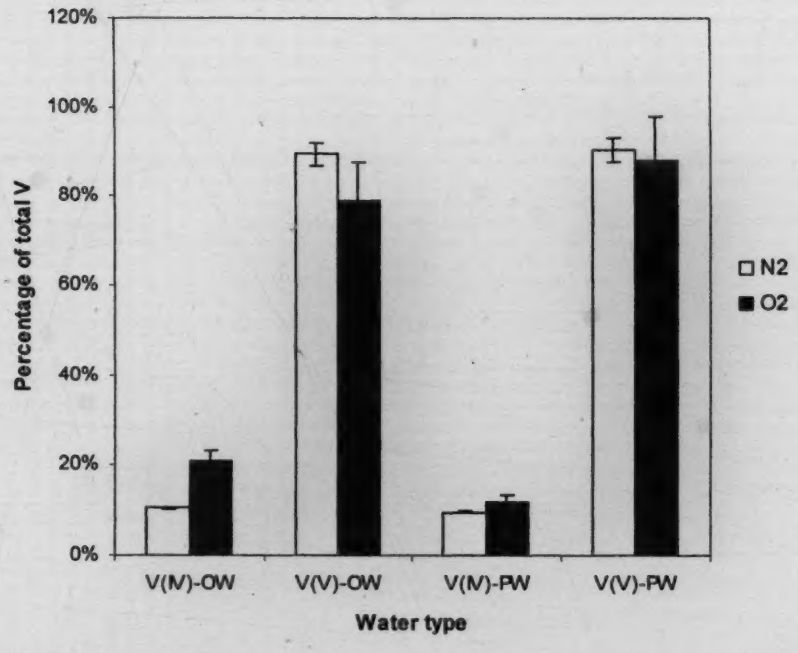

Figure 3-17. Effect of DO level on V speciation in SAC. OW denotes overlying water and PW denotes pore water. The error bars denote standard deviation of three experiments

Again, no statistically significant difference was found between samples derived from the two treatments in SAC.

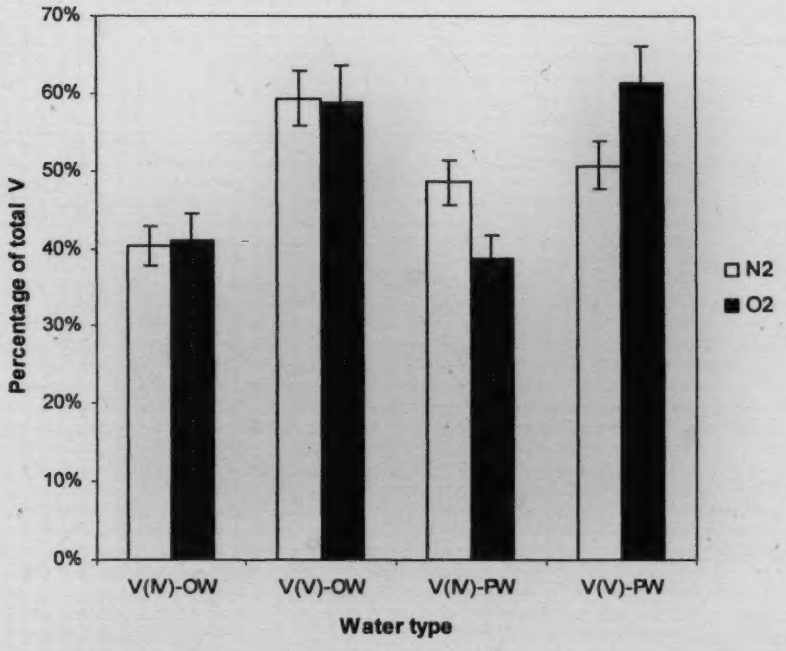

Figure 3-18. Effect of DO level on V speciation in SDC. OW denotes overlying water and PW denotes pore water. The error bars denote standard deviation of three experiments

According to t-Tests done to compare each set of sample, no significant differences are found between the two treatments.

In conclusion to the pH and DO experiments, as pH increases, there is less V(IV) and more V(V) in the OW and PW. The effect of DO is not as apparent as pH on V speciation. In fact, in all the DO experiments, there are no differences of V speciation between the O_2-treatment and N_2-treatment in neither the overlying water nor the pore water.

3.2 V(V)-Incubated Shewanella putrefaciens *CN32*

3.2.1 Background

In the U.S., ores in the Colorado Plateau region were mined for vanadium and uranium. An important ore is carnotite, or $K_2(UO_2)_2(VO_4)_2$. Carnotite is a bright greenish yellow mineral that commonly occurs as coatings on sandstone. It contains both V(V) and U(VI) in a mineral lattice. Tailings from V and U mining, chiefly in the form of carnotite, were disposed at processing sites near Rifle, Colorado. The tailing piles were removed as a remedial measure to reduce groundwater levels of U, V and other toxic metals; groundwater at this site flows towards the Colorado River to the immediate south. However, subsurface plumes enriched in U and V continue to migrate.

The bacterium *S. putrefaciens* CN32 was isolated from the same geographic region where carnotite was mined. *S. putrefaciens* CN32 is a facultatively anaerobic bacteria commonly found in natural aquatic and sedimentary environments (7). It is capable of reducing a number of metals including Cr(VI), Mn(IV), Fe(III), U(VI) and Tc(VII), but it is not yet known whether it could also reduce V(V) (8).

Recent studies have shown that a related species, *S. oneidensis* MR-1, is able to reduce V(V) to V(IV) (9), but no studies have been reported as to whether *S. putrefaciens* CN32 would have the same ability.

3.2.2 Overview of experiments

To see if *S. putrefaciens* CN32 could reduce V(V), the bacterium was incubated with V(V) in forms of soluble sodium metavanadate (SMV) and insoluble carnotite (CARN) in serum bottles containing liquid media. The bottles were handled in an controlled atmosphere chamber containing 5% H_2:95% Ar. All manipulations of the

samples, including packaging the samples for shipping, were conducted in the chamber. To determine V speciation in the soluble fraction, individual treatments were sampled at intervals from the serum bottles using sterile syringes and filtered to remove the bacteria/mineral solid. EDTA (1.5 mM) was added to 1.0mM of total V. The filtrate samples were shipped to our laboratory immediately under H_2/Ar in serum bottles sealed with thick butyl rubber stoppers and analyzed as soon as possible to avoid oxidation.

Later in the study, unfiltered samples of the cultured bacteria were shipped to the laboratory and the sample separated into the filtrate, cell wall wash and cell lysate fractions for analysis. This was done to help interpret how and where the bioreduction, if any, was taking place.

3.2.3 Analysis of bacterial filtrate

In order to assess whether *S. putrefaciens* CN32 has the potential for V bioreduction, anaerobic cultures of the bacteria were incubated with vanadate as the sole electron acceptor in a chemically defined minimal medium containing components displayed in Table 3-8.

Table 3-8. Components of the minimal defined culture medium. NTA stands for nitrilotriacetate monohydrate and PIPES is piperazine-1,4-bis(2-ethanesulfonic acid)

Components	M.W. (g/mol)	Conc. (M)	Wt./100x Conc. (g/L)
Na lactate	112.1	1.8×10^{-3}	N/A
NH_4Cl_2	53.5	2.2×10^{-3}	11.76
KCl	74.6	1.2×10^{-3}	8.95
$CaCl_2$	111.0	6.1×10^{-4}	6.77
NTA x H_2O	257.1	7.1×10^{-5}	1.83
$MgSO_4$ x $7H_2O$	246.5	1.1×10^{-4}	2.71
NaCl	58.4	1.5×10^{-4}	0.88
$MnSO_4$ x H_2O	169.0	2.7×10^{-5}	0.46
$ZnCl_2$	136.8	8.6×10^{-6}	0.12
$FeSO_4$ x $7H_2O$	278.0	3.2×10^{-6}	0.089
$CaCl_2$ x $2H_2O$	147.0	6.1×10^{-6}	0.090
$CoCl_2$ x $6H_2O$	237.9	3.8×10^{-6}	0.090
Na_2MoO_4 x $2H_2O$	242.0	9.3×10^{-7}	0.023
Na_2WO_4 x $2H_2O$	330.0	6.8×10^{-7}	0.022
$NiCl_2$ x $6H_2O$	237.7	9.1×10^{-7}	0.022
$CuSO_4$ x $5H_2O$	249.7	3.6×10^{-7}	0.009
$AlK(SO_4)_2$ x $12H_2O$	474.4	1.9×10^{-7}	0.009
H_3BO_3	61.8	1.5×10^{-6}	0.009
NaH_2PO_4 x H_2O	138.0	3.9×10^{-3}	53.82
$NaHCO_3$	84.0	2.7×10^{-2}	226.83
PIPES	335.4	4.5×10^{-3}	151.0

3.2.3.1 SMV-incubated bacterial filtrate

SMV was provided to the bacteria as a 4 mM solution initially. Due to concerns regarding the toxicity of SMV to the bacteria, it was administered as a 2 mM solution after the first dose.

The samples taken after 1-day, 54-day and 120-day incubation periods were investigated. The 1-day and 54-day samples were done in two replicates (a and b) and the control samples did not contain any bacteria. The filtrates of the samples were separated from the bacteria by filtering through a 0.2 μm filter and analyzed by HPLC-ICPMS. Figure 3-19 shows the SMV-incubated bacteria sampled at different times.

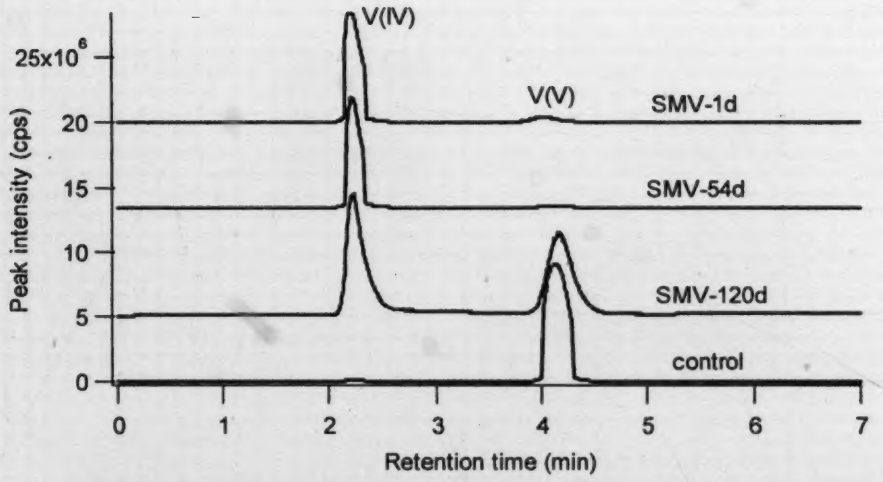

Figure 3-19. V speciation in filtrates extracted from *S. putrefaciens* CN32 incubated with sodium metavanadate. Chromatograms were manually shifted on the vertical axis to show the reduction process. The actual baselines were the same among the chromatograms

From the first two chromatograms in Figure 3-19, it is seen that most V(V) was reduced to V(IV) after 1 day, and nearly all V(V) is reduced over the course of 54 days. V(IV) is then gradually oxidized to V(V) over a longer period of time. Some of the bacteria were still viable by the 120th day and the experiment was done in an anaerobic environment, thus it is not entirely clear what caused the oxidation. It is likely that traces of O_2 and organic material released by the bacteria as they lysed were responsible for the oxidation of V(IV) (10). At the beginning, when there were plenty of bacteria, V was likely re-reduced quickly once it was oxidized. As the cultures started to die out, the rate of oxidation exceeded reduction.

Table 3-9. Concentrations of V species in filtrates extracted from *S. putrefaciens* CN32 incubated with sodium metavanadate[a]. Duplicates are denoted with a and b, ctrl denotes the control sample which does not contain any bacteria

Sample	*V(III) (mg/L)*	*V(IV) (mg/L)*	*V(V) (mg/L)*
SMV a – 1day	BDL	94	23
SMV b – 1day	0.083	120	1.0
SMV a – 54day	0.061	72	0.70
SMV b – 54day	0.11	95	1.0
SMV – 120day	BDL	37	12
SMVcontrol	BDL	39	96

[a]Total concentrations of V are not equal between each sample because the samples were extracted out of a nonhomogeneous mixture.

Table 3-10. Percentages[b] of V species in filtrates extracted from *S. putrefaciens* CN32 incubated with sodium metavanadate. Duplicates are denoted with a and b, ctrl denotes the control sample which does not contain any bacteria

Sample	*% V(III)*	*% V(IV)*	*% V(V)*
SMV a – 1day	BDL	80.0	19.7
SMV b – 1day	0.1	98.7	0.8
SMV a – 54day	0.1	97.9	0.9
SMV b – 54day	0.1	98.1	1.1
SMV – 120day	BDL	74.8	24.2
SMV ctrl	BDL	28.7	71.3

[b]Percentages are calculated based on peak areas.

From the data presented in Table 3-9 and Table 3-10, there was a larger percentage of V(IV) than V(V) in all the samples compared to the control. This implies that *S. putrefaciens* CN32 was able to reduce vanadium in its +5 oxidation state to its major reduced product V(IV). Besides the V(IV) and V(V) peaks, a small peak was also observed around 0.83 min. This peak was later identified as V(III) and it was observed at 0.1% of the total V concentration in three of the samples. In the 120-d sample, all of the V(III) was oxidized to V(IV) and some V(IV) was oxidized to V(V). The presence of some V(IV) in the control sample indicates that there is probably some abiotic reduction, possibly by Fe(II) and the PIPES buffer which is a weak reductant in the culture medium.

3.2.3.2 CARN-incubated bacterial filtrate

CARN is a naturally occurring mineral that contains V(V). It was incubated in its solid form with the bacteria. There were six samples – two 1-day samples (replicates a and b), two 54-day samples, one 120-day sample and one control sample containing no bacteria. The filtrate of the samples were isolated from the mineral by filtering through a 0.2 μm filter and analyzed by HPLC-ICPMS.

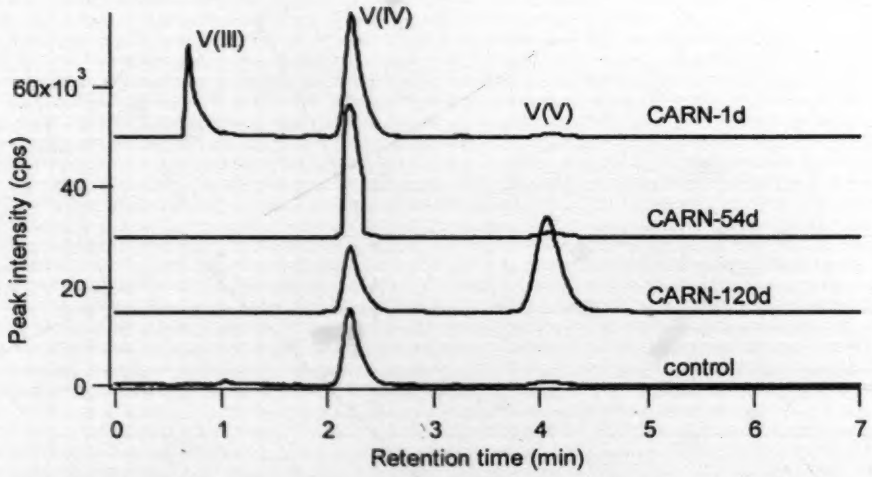

Figure 3-20. V speciation in filtrate from carnotite incubated cultures. Chromatograms were manually shifted on the vertical axis to show the reduction process. The actual baselines were the same among the chromatograms

In the 1-day sample (Figure 3-20), a very sharp peak at 0.83 min was observed. After approximately two months, this sample was analyzed again and the peak at 0.83 min disappeared and the V(IV) peak increased (Figure 3-21), an indication that the peak at 0.83 min is probably due to V(III).

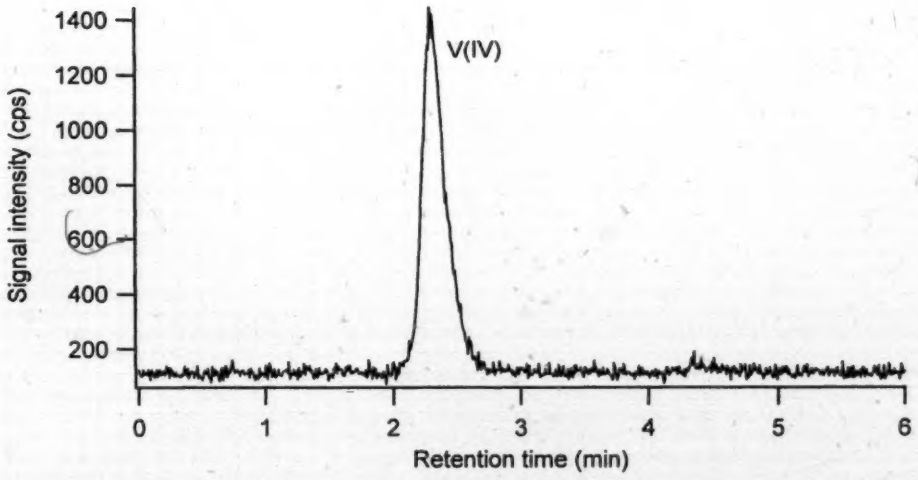

Figure 3-21. Carnonite-1d filtrate sample analyzed two months after receipt

Another observation that supports this conclusion was that when the bacterium was initially transferred to the medium containing carnotite, a green color started to appear within a couple of hours (11). Since the V(III) ion appears green in solution, this color is most likely due to its presence. By the 120th day, some of the V(IV) peak was oxidized to V(V).

To confirm the peak at 0.83 min is indeed due to V(III), a standard was made up using the procedure outlined in Section 2.2 and it was analyzed on the HPLC-ICPMS.

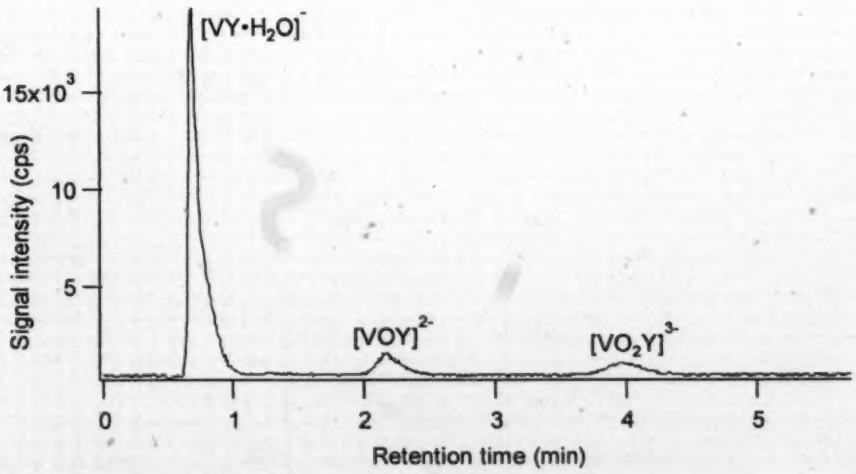

Figure 3-22. $[VY{\cdot}H_2O]^-$ (250 µg/L) after 5 min of make up

As shown in Figure 3-22, some of the V(III) was oxidized to V(IV) and V(V) shortly after dilution. The retention time of the V(III) standard matched that of the peak found in the sample. Thus the peak at 0.83 min was indeed V(III).

Table 3-11. V speciation in carnotite incubated *S. putrefaciens* CN32. Duplicates are denoted with a and b, ctrl denotes the control sample which does not contain any bacteria

Sample	V(III) (mg/L)	V(IV) (mg/L)	V(V) (mg/L)
CARN a – 1day	1.7	1.85	0.016
CARN b – 1day	1.4	1.48	0.029
CARN a – 54day	0.16	110	1.2
CARN b – 54day	0.57	130	0.24
CARN – 120day	0.34	63	1.32
CARN ctrl	BDL	1.0	0.038

Table 3-12. Percentage of V in carnotite incubated *S. putrefaciens* CN32. Duplicates are denoted with a and b, ctrl denotes the control sample which does not contain any bacteria

Sample	% V(III)	% V(IV)	% V(V)
CARN a – 1day	47.8	51.8	0.4
CARN b – 1day	47.6	51.4	1.0
CARN a – 54day	0.1	98.4	1.0
CARN b – 54day	0.5	99.2	0.2
CARN – 120day	0.5	97.4	2.1
CARN ctrl	BDL	93.2	3.7

According to Table 3-11 and Table 3-12, almost 50% of V was in the form of V(III) in the 1-day incubated carnotite samples. Within 54 days, most of the V(III) oxidized to V(IV). After 120 days, some of the V(IV) was oxidized to V(V), but most of the V remains as V(IV). In the control sample, V(IV) was still the major species, which indicates that there is major abiotic reduction taking place in the carnotite incubated samples. However, V(IV) does not further reduce to V(III).

3.2.4 Analysis of bacterial fractions

To better understand the reduction process used by *S. putrefaciens* CN32, V-incubated bacteria was separated into three fractions – filtrate containing soluble V after respiration, cell wall wash containing any V at the cell wall, and bacterial lysate containing any V the bacteria has taken up through sorption or transport mechanisms.

The samples analyzed were incubated with SMV or CARN for 138 days. They consist of two replicates and one control for each type of sample. The control samples do not contain any bacteria. Upon receipt of the samples, excess EDTA was added to all the samples. Two milliliters of each sample were centrifuged to separate a filtrate layer and a solid containing bacteria and mineral at the bottom. The supernatant was carefully collected using a glass pipette. The bacteria/mineral solid was washed twice with 100 µL Na_3PO_4 (10 mM) solution at pH 6 to maintain the structural integrity of the cells. The bacteria wash fractions were collected and analyzed. To the remaining bacteria/mineral solid, 1 mL of DIW was added and the cells sonicated for 0.5 h without heating to lyse all the bacteria. The supernatant was filtered through a 2 µm syringe filter and analyzed. Table 3-13 and Table 3-14 summarize the concentrations and percentages of each V species observed in each fraction.

Table 3-13. V speciation in the filtrate fraction of *S. putrefaciens* CN32 incubated with SMV or CARN for 138 days. Duplicates are denoted with a and b, ctrl denotes the control sample which does not contain any bacteria

Sample	V(III) (mg/L)	V(IV) (mg/L)	V(V) (mg/L)
SMV a	BDL	7.9	1.8
SMV b	0.090	7.0×10^2	3.5
SMV ctrl	BDL	43	110
CARN a	BDL	750	15
CARN b	BDL	1100	0.79
CARN ctrl	BDL	4.5	1.2

It seems that after 138 days, there is no V(III) left in the CARN samples. Most of V remained in the form of V(IV) in all the samples, as can be seen in Table 3-13. Comparing SMV ctrl and CARN ctrl, there seems to be more abiotic reduction with bacteria incubated with CARN since there is more V(IV) there.

Table 3-14. Percentages of V species in filtrate fraction of *S. putrefaciens* CN32 incubated with SMV or CARN for 138 days. Duplicates are denoted with a and b, ctrl denotes the control sample which does not contain any bacteria

Sample	% V(III)	% V(IV)	% V(V)
SMV a	BDL	81.2	18.8
SMV b	BDL	99.5	0.5
SMV ctrl	BDL	29.0	71.0
CARN a	BDL	98.0	2.0
CARN b	BDL	99.9	0.1
CARN ctrl	BDL	79.1	20.9

Unlike for the filtrate samples, V(III) is found in three out of the four samples incubated with the bacteria in the first bacterial cell wall wash fraction. This could mean that the environment at the cell wall is quite reducing. Data from Table 3-15 to Table 3-18 show the distribution of V in the first two washes of the cell wall.

Table 3-15. V speciation in the first bacteria wall wash fraction of *S. putrefaciens* CN32 incubated with SMV or CARN for 138 days. Duplicates are denoted with a and b, ctrl denotes the control sample which does not contain any bacteria

Sample	V(III) (mg/L)	V(IV) (mg/L)	V(V) (mg/L)
SMV a	0.10	11	0.18
SMV b	0.27	16	0.27
SMV ctrl	BDL	1.5	3.23
CARN a	BDL	20	3.22
CARN b	0.030	29	1.17
CARN ctrl	BDL	1.9	0.53

Table 3-16. Percentages of V species in first bacteria cell wall wash fraction of *S. putrefaciens* CN32 incubated with SMV or CARN for 138 days. Duplicates are denoted with a and b, ctrl denotes the control sample which does not contain any bacteria

Sample	% V(III)	% V(IV)	% V(V)
SMV a	0.9	97.6	1.6
SMV b	1.6	96.8	1.6
SMV ctrl	BDL	32.3	67.7
CARN a	BDL	86.2	13.8
CARN b	0.1	96.0	3.9
CARN ctrl	BDL	78.6	21.4

Table 3-17. V speciation in the second bacteria wall wash fraction of *S. putrefaciens* CN32 incubated with SMV or CARN for 138 days. Duplicates are denoted with a and b, ctrl denotes the control sample which does not contain any bacteria

Sample	V(III) (mg/L)	V(IV) (mg/L)	V(V) (mg/L)
SMV a	0.0080	2.3	0.092
SMV b	0.060	4.3	BDL
SMV ctrl	BDL	0.33	0.13
CARN a	BDL	5.7	0.30
CARN b	BDL	7.7	0.53
CARN ctrl	BDL	0.64	0.11

Table 3-18. Percentages of V species in second bacteria cell wall wash fraction of *S. putrefaciens* CN32 incubated with SMV or CARN for 138 days. Duplicates are denoted with a and b, ctrl denotes the control sample which does not contain any bacteria

Sample	% V(III)	% V(IV)	% V(V)
SMV a	0.3	95.8	3.9
SMV b	1.4	98.6	BDL
SMV ctrl	BDL	72.1	27.9
CARN a	BDL	94.9	5.1
CARN b	BDL	93.5	6.5
CARN ctrl	BDL	85.9	14.1

As expected, the first wash of the bacteria cell wall (Table 3-15) contained more V than the second (Table 3-17). However, V(III) still persisted after the second wash (Table 3-17). This could mean that the reduction of V(V) occurs at the cell wall. The absence of V(III) in the filtrate probably indicates that V(III) was oxidized once it leaves the cell wall. The last fraction analyzed was the bacteria lysate and the speciation data for this fraction is shown in Table 3-19 and Table 3-20.

Table 3-19. V speciation in the bacterial lysate fraction of *S. putrefaciens* CN32 incubated with SMV or CARN for 138 days. Duplicates are denoted with a and b, ctrl denotes the control sample which does not contain any bacteria

Sample	V(III) (mg/L)	V(IV) (mg/L)	V(V) (mg/L)
SMV a	BDL	0.47	0.13
SMV b	0.020	0.54	0.013
SMV ctrl	BDL	BDL	BDL
CARN a	BDL	0.63	0.46
CARN b	BDL	1.1	0.30
CARN ctrl	BDL	0.26	0.071

Again the controls are V(V) in cell medium without any bacteria, thus they contain little or no V. The minimal amount of V observed in the CARN control is probably due to the remaining undissolved mineral. In all cases, there is a minute amount of V in the samples, indicating that the bacteria have taken in none or very little V.

Table 3-20. Percentages of V species in bacteria lysate fraction of *S. putrefaciens* CN32 incubated with SMV or CARN for 138 days. Duplicates are denoted with a and b, ctrl denotes the control sample which does not contain any bacteria

Sample	% V(III)	% V(IV)	% V(V)
SMV a	BDL	78.7	21.3
SMV b	3.5	94.1	2.4
SMV ctrl	BDL	BDL	BDL
CARN a	BDL	57.5	42.5
CARN b	BDL	78.7	21.3
CARN ctrl	BDL	78.8	21.2

From the analysis of the bacterial fractions, we can infer that reduction probably occurs at the cell wall, since V(III) is observed at higher concentrations there than anywhere else in the bacteria. This hypothesis was confirmed by transmission electron micrograph (TEM) data shown in Figure 3-23.

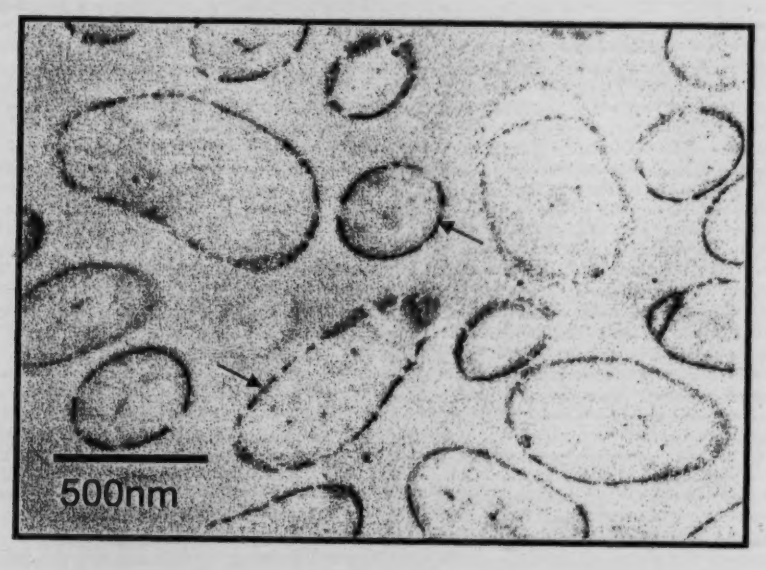

Figure 3-23. TEM of bacteria culture in thin section after respiring sodium metavanadate under anaerobic conditions for 3 days. Precipitates at the cell wall are indicated by arrows. Courtesy of Dr. Glasauer, University of Guelph

Upon close examination, the periplasm, the protein-rich gel-like space between the outer and cytoplasmic membranes, is encrusted with fine precipitates of vanadium about 2 nm in diameter. These precipitates are likely due to V(IV), since it is the major observed species of V between 1 and 54 days (Figure 3-19). This is further supported by the fact that V(IV) is less soluble than V(V) (12).

In an X-ray spectrum (Figure 3-24) taken from the cell wall by energy dispersive spectroscopy, which detects the presence of precipitates at a resolution of around 100 nm, several metals are observed including vanadium. This confirms that V is in fact present

at the cell wall. P in the spectrum is an integral part of the cell; the Cu signal is due to the Cu support grid used for the samples and the C signal arises from the film used to coat the support grid as well as from the cell.

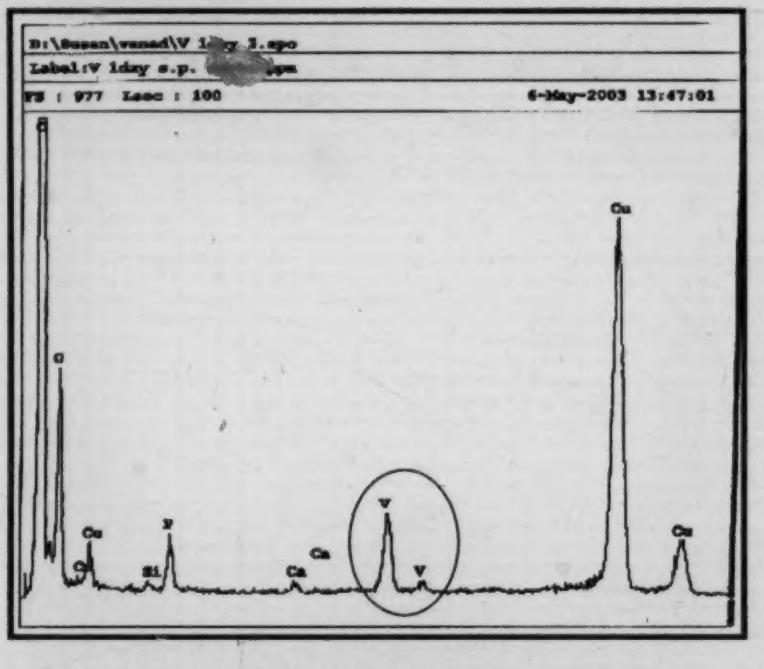

Figure 3-24. Energy dispersive spectroscopy shows that the cell wall contains V. Courtesy of Dr. Glasauer, University of Guelph

It has been shown before that *S. putrefaciens* CN32 can reduce soluble or mineral bound Fe(III) by a dissimilatory mechanism in which Fe(III) serves as the electron acceptor for the membrane bound electron transport chain during respiration (13). Since the reduced form of V is observed at the cell membrane, it could well be that V(V) acts as the electron acceptor at the cell wall by a similar mechanism.

Gram negative bacteria such as *S. putrefaciens* CN32 have an abundance of lipopolysaccharide at their cell wall, which consists of lipids and carbohydrates that maintain the structural integrity of the bacteria (10). The carboxyl and phosphoryl groups of lipopolysaccharide make the bacteria highly negatively charged and therefore they

possess a high affinity for cationic metal species (14). Soluble metals such as Fe(III) are attracted to the cell wall of *S. putrefaciens* CN32 due to electrostatic interactions. The cell wall of the bacteria could also have an affinity for V(V) by similar interactions.

In conclusion, *S. putrefaciens* CN32 is able to reduce the soluble SMV to mostly V(IV) in one day. It is also able to reduce the insoluble mineral CARN to V(IV) as well as V(III) in one day. This is the first time that *S. putrefaciens* CN32 has been observed to reduce V. With this bacteria, vanadium is not only reduced to its common form of V(IV), but it is also observed to reduce to the less stable V(III). The reduction process most likely occurs at the cell wall of the bacteria, where V(V) is reduced to fine precipitates of V(IV). Over time, some of the V(IV) and most of the V(III) are oxidized in the bacterial filtrate. These findings are exciting since V(III) and V(IV) are less toxic than V(V), and by reducing V(V) to V(IV) at the bacterial surface, the soluble and more toxic V(V) in the environment can possibly be immobilized and properly disposed.

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Chapter 4. Conclusions and Future Research

4.1 Conclusions

Even though vanadium has often been a neglected heavy metal under study, it is an emerging contaminant in the environment, especially in industries associated with oil production. It is toxic to environmental and biological systems when it is present at mg/L concentrations. In addition, V(V) is a more toxic species than V(IV) and V(III).

A HPLC-ICPMS method was developed for the simultaneous speciation of the three species of vanadium as EDTA complexes on a SAX column. The optimum mobile phase contained 3% ACN, 2 mM EDTA and 80 mM ammonium bicarbonate at pH 6. Each analysis was complete in about 5 min with good resolution and sensitivity. There were no significant matrix effects observed when the method was applied to coke water and bacterial samples. Due to its short analysis time and high sensitivity, this simple method can be applied to routine analysis of samples containing vanadium.

4.1.1 Coke Pore Waters

To test the robustness of the method, it was first applied to coke water samples from Syncrude Canada Ltd. The only species of V observed were V(IV) and V(V) in these samples. In most of the field samples, the observed V is in the form of V(V). The V(V) concentration was found to be as high as 5.2 mg/L at the deep capped lysimeter site in the field, which highly exceeds the 3 μg/L usually contained in natural waters (1). This concentration also exceeds the LC_{50} values for some aquatic organisms such as the guppy and goldfish. Depending on the species of V administered, the 6-d LC50 values were 0.9-4.5 mg/L for goldfish and 0.12-0.65 mg/L for guppy (2).

As expected by intuition and confirmed by experimentation, more water leaches out more V from coke and the concentration of the metal is higher at sites containing a smaller volume of water. It does not take long to leach V out of coke, most of it is often extracted with just three extractions. On the reclamation lands, coke should be isolated from water sources as much as possible to avoid chronic leaching of the metal.

From leaching studies done with a salt solution, it was found that $[VO_2Y]^{3-}$ binds with divalent cations Mg^{2+} and Ca^{2+} via polar interactions, the interactions are especially significant with Ca^{2+}. From the literature, it is known that V(V) interacts with Zn^{2+} via polar interactions (3), so it probably also interact with Ca^{2+} the same way. These findings could be useful in revealing how V(V) acts in place of phosphate in biological systems, which is a poorly understood process.

Continuing with the leaching studies, three types of coke were leached and compared. SFC contains the most amount of V since it has not been in contact with water. SAC and SDC contain about the same amount of V, but the former is much more oxidized, since it was slurried with water for transportation purposes.

Under conditions with higher pH, less V(IV) and more V(V) was observed since V is oxidized at higher pH. The DO experiments reveal that there is generally more V(IV) and less V(V) under anoxic conditions. SAC is an exception since it has already been extensively oxidized.

Even though the production of coke cannot be avoided altogether, these studies will hopefully help us better understand the properties of coke, thus enabling us to make more informed decisions regarding where and how to store the potentially useful coke in order to minimize its toxic effects and environmental risks.

4.1.2 *Shewanella putrefaciens* CN32

The second type of samples the method was tested on were V(V)-incubated *Shewanella putrefaciens* CN32 samples. The objective was to see whether *S. putrefaciens* CN32 has the ability to reduce V(V) to the lower oxidation states, or less toxic forms of V.

Vanadium in its fifth oxidation state was administered as either the soluble SMV or the mineral CARN. When *S. putrefaciens* CN32 was incubated with SMV, it reduced most of the V(V) to V(IV) in just one day. When the sample was analyzed on the 54th day, V(IV) remained in its reduced form. By the 120th day, some of the V(IV) oxidized to V(V). Due to the presence of V(IV) in the control sample, it can be concluded that there is probably abiotic reduction taking place.

When incubated with carnotite, the bacterium was not only able to reduce V(V) to V(IV), but to a significant amount of V(III) as well. However, V(III) was not stable in the medium and was oxidized to V(IV) in 54 days. By the 120th day, some of the V(IV) oxidized to V(V). Again, abiotic reduction is observed in the carnotite incubated bacteria samples, and this is probably due to the presence of Fe(II) and a reducing buffer in the incubation medium.

To answer the initially posed research question, *S. putrefaciens* CN32 is able to reduce V(V) in the forms of SMV and CARN to lower oxidation states of V. SMV was reduced mostly to V(IV) whereas CARN was reduced to V(IV) and V(III).

From analyzing the bacteria filtrate, cell wall washes and bacterial lysate fractions, V(III) was observed mostly in the cell wall wash fraction. This indicates that the reduction probably occurs at the cell wall of the bacteria. This supposition is

corroborated by supporting data from transmission electron micrograph and energy dispersive spectroscopy provided by Dr. Glasauer from the University of Guelph.

4.2 Future Work

The conclusions from the bacteria studies are interesting because *S. putrefaciens* CN32 was found to possess the capability to reduce the more toxic V(V) to less toxic and less soluble forms of V. This property could be used as a bioremediation technique at environmental sites contaminated with V(V) in the future. To test this possibility, *S. putrefaciens* CN32 could be introduced to a body of filtered coke water containing V(V). Once V is reduced in a couple of days, the bacteria would be coated with insoluble V(IV), and potentially V(III). The vanadium-encrusted bacteria could then be filtered from the coke water. If the bacteria are still viable at this point, V can potentially be washed away by treating the culture with oxic water. V(IV) will then oxidize back to V(V) and the waste water can then be disposed of properly. The bacteria can then be reused for further reduction of the metal. This process can also be applied to other metals such as chromium, for which the (VI) oxidation state is more toxic than the (III) and is more soluble (4).

In the coke pore waters collected from the field, the metal present at the second highest concentration is molybdenum. At the deep capped lysimeter site, Mo is found around 2-4 mg/L (5). Mo(V) and (VI) are known to form stable complexes with EDTA (6) and thus could potentially be simultaneously separated from the species of V by modifying the current method using HPLC-ICPMS in the multi-element detection mode. Even though Mo is known to have a low toxicity, molybdenum (VI) was found to inhibit the growth of the bacteria *Thiobacillus thiooxidans* ATCC 8085 (7). Further studies in

Mo speciation could be useful in assessing the effect of this metal on the environment and biological systems.

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